Ecological Speciation

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1. Introduction

Ecological speciation occurs when adaptation to divergent environments, such as different resources or habitats, leads to the evolution of reproductive isolation [1, 2]. More specifically, divergent (or disruptive) selection between environments causes the adaptive divergence of populations, which leads to the evolution of reproductive barriers that decrease, and ultimately cease, gene flow [3, 4]. Supported by a growing number of specific examples, ecological speciation is thought to be a primary driving force in evolutionary diversification, exemplified most obviously in adaptive radiations [5–8].

As acceptance of the importance of ecological speciation has grown, so too has the recognition that it is not all powerful. Specifically, a number of instances of nonecological speciation and nonadaptive radiation seem likely [9], and colonization of different environments does not always lead to speciation [10, 11]. This latter point is obvious when one recognizes that although essentially all species are composed of a number of populations occupying divergent environments [12], only a fraction of these ever spin off to become full-fledged species. Instead, populations occupying divergent environments or using different resources show varying levels of progress toward ecological speciation—and this variation provides the substrate to study factors that promote and constrain progress along the speciation continuum. By studying these factors, we can begin to understand why there are so many species [13] and also why there are so few species [14].

This special issue on ecological speciation puts snapshots of progress toward speciation sharply in focus and then investigates this topic from several angles. First, several papers provide conceptual or theoretical models for how to consider progress toward ecological speciation (Funk; Heard; Lenormand; Liancourt et al.; Agrawal et al.). Second, several papers highlight the noninevitability of ecological speciation through investigations where ecological speciation seems to be strongly constrained (Räsänen et al.; Bolnick) or at least lacking definitive evidence (Ostevik et al.; Scholl et al.). Some of these papers also uncover specific factors that seem particularly important to ecological speciation, such as the combination of geographic isolation and habitat differences (Surget-Groba et al.), the strength of disruptive selection and assortative mating (Bolnick), and host-plant adaptation (Scholl et al.). Third, several particularly important factors emerge as a common theme across multiple papers, particularly parasites/pollinators (Xu et al.; Karvonen and See-hausen), habitat choice (Webster et al.; Feder et al.; Carling and Thomassen; Egan et al.), and phenotypic plasticity (Fitzpatrick; Vallin and Qvarnström).

Here we highlight the most important aspects of these contributions and how they relate to three major topics: (i) models for progress toward ecological speciation; (ii) variable progress toward ecological speciation in nature; and (iii) factors affecting progress toward ecological speciation.
2. Models for Progress toward Ecological Speciation

Terminological issues have long bedevilled communication among researchers working on speciation. D. J. Funk addresses this topic by first clarifying the relationship between sympatric speciation (whereby reproductively isolated populations evolve from an initially panmictic population) and ecological speciation (whereby reproductive isolation evolves as a consequence of divergent/disruptive natural selection). These are orthogonal concepts [15]. First, even if disruptive selection is a common way of achieving sympatric speciation, this can also be caused by other factors, such as changes in chromosome number. Second, ecological speciation can readily occur in allopatry [16, 17]. Funk then introduces four new concepts aiming to reduce confusion in the literature. *Sympatric race* is a generalisation of *host race* (usually used for herbivores or parasites) and refers to any sympatric populations that experience divergent selection and are partly but incompletely reproductively isolated. *Envirotypes* are populations that differ due to phenotypic plasticity. *Host forms* are populations that exhibit host-associated variation, but for which the nature of variation (e.g., envirotype, host race, cryptic species) has not yet been diagnosed. *Ecological forms* are a generalization of *host forms* for nonherbivore or parasitic taxa. The two latter concepts acknowledge the fact that one has an incomplete understanding of speciation. To overcome the problem of overdiagnosing host races, Funk introduces five criteria, based on host association and choice, coexistence pattern, genetic differentiation, mate choice, gene flow, and hybrid unfitness. Funk’s maple and willow associated phytophagous populations of *Neochlamisus bebianaec* leaf beetle meet all these criteria and can, therefore, be considered as host races.

Another phytophagy-inspired conceptual model for how an insect species initially using one plant species might diversify into multiple insect species using different host plants is presented by S. Heard. This effort explicitly links variation in host plant use within insect species or races to the formation of different host races and species. In this proposed “gape-and-pinchn” model, Heard posits four stages (or “hypotheses”) of diversification defined in part by overlap in the plant trait space used by the insect races/species. In the first stage “adjacent errors,” some individuals within an insect species using one plant species might “mistakenly” use individuals of another plant species that have similar trait values to their normal host plant species. In the next stage “adjacent oligophagy,” populations formed by the insects that shifted plant species then experience divergent selection—and undergo adaptive divergence—leading to a better use of that new host. In the third stage “trait distance-divergence,” competition and reproductive interactions cause character displacement between the emerging insect races or species so that they become specialized on particularly divergent subsets of the trait distributions of the two plant species. In the final stage “distance relaxation,” the new species become so divergent that they no longer interact, and can then evolve to use trait values more typical of each plant species. Heard provides a theoretical and statistical framework for testing this model and applies it to insects using goldenrod plants.

Local adaptation is often the first step in ecological speciation, and so factors influencing local adaptation will be critical for ecological speciation. Local adaptation can either increase over time (if more specialized alleles spread), eventually leading to speciation, or it can decrease over time (if more generalist alleles spread). T. Lenormand reviews the conditions that favor these different scenarios and emphasizes the role of three positive feedback loops that favor increased specialization. In the demographic loop, local adaptation results in higher population density, which in turn favors the recruitment of new locally adapted alleles. In the reinforcement loop, locally adapted alleles are more likely to be recruited in genomic regions already harbouring loci with locally adapted alleles, thereby generating genomic regions of particular importance to local adaptation. In the reinforcement loop, local adaptation selects for traits that promote premating isolation (reinforcement), which in turn increases the recruitment and frequency of locally adapted alleles. Lenormand then details the mechanisms involved in reinforcement, particularly assortative mating, dispersal, and recombination. He highlights that these characteristics represent the three fundamental steps in a sexual life cycle (syngamy, dispersal, and meiosis) and that they promote genetic clustering at several levels (within locus, among individuals, among loci). His new classification is orthogonal to, and complements, the traditional one-versus two-allele distinction [14]. Overall, the rates of increased specialization and reinforcement determine progress toward ecological speciation.

One of the major constraints on ecological speciation is the establishment of self-sustaining populations in new/marginal environments, because the colonizing individuals are presumably poorly adapted to the new conditions. This difficulty might be eased through facilitation, the amelioration of habitat conditions by the presence of neighbouring living organisms (biotic components) [18]. According to this process, the benefactor’s “environmental bubble” facilitates the beneficiary’s adaptation to marginal conditions, which can result in ecological speciation if gene flow from the core habitat is further reduced. At the same time, however, facilitation might hinder further progress toward ecological speciation by maintaining gene flow between environments and by preventing reinforcement in secondary contact zones. P. Liancourt, P. Choler, N. Gross, X. Tibert-Plante, and K. Tielbörger consider these possibilities from the beneficiary species perspective, through a spatially and genetically explicit modelling framework that builds on earlier models [19, 20]. They find that ecological speciation is more likely with larger patch (facilitated versus harsh) sizes. Liancourt and coauthors further suggest that facilitation can play another important role in evolution by helping to maintain a genetic diversity “storage” in marginal habitats, a process with some parallel to niche conservatism. A deeper understanding of the role of facilitation in diversification is needed (both theoretically and empirically), and the authors suggest that stressful environmental gradients would be useful study systems for this endeavour.
Intrinsic postzygotic isolation, a fundamental contributor to speciation, is often caused by between-locus genetic incompatibilities [21, 22]. The origin of these incompatibilities, particularly in the face of gene flow, remains an outstanding question. A. F. Agrawal, J. L. Feder, and P. Nosil use two-locus two-population mathematical models to explore scenarios where loci subject to divergent selection also affect intrinsic isolation, either directly or via linkage disequilibrium with other loci. They quantified genetic differentiation (allelic frequencies of loci under selection), the extent of intrinsic isolation (hybrid fitness), and the overall barrier to gene flow (based on neutral loci). They find that divergent selection can overcome gene flow and favors the evolution of intrinsic isolation, as suggested previously [23]. Counterintuitively, intrinsic isolation can sometimes weaken the barrier to gene flow, depending on the degree of linkage between the two focal loci. This occurs because intrinsic isolation sometimes prevents differentiation by divergent selection.

3. Variable Progress toward Ecological Speciation in Nature

Three-spine stickleback fish, with their diverse populations adapted to different habitats, had provided a number of examples of how adaptive divergence can promote ecological speciation [24, 25]. Indeed, work on this group has fundamentally shaped our modern understanding of ecological speciation [1–3]. At the same time, however, three-spine stickleback also provides evidence of the frequent failure of divergent selection to drive substantial progress toward ecological speciation [25]. This special issue provides two of such examples. In one, K. Räsänen, M. Delcourt, L. J. Chapman and A. P. Hendry report that, despite strong divergent selection, lake and stream stickleback from the Misty watershed do not exhibit positive assortative mate choice in laboratory experiments. These results are in contrast to the strong assortative mating observed in similar studies of other stickleback systems, such as benthic versus limnetic [26] and marine versus fresh water [27]. In addition to providing potential explanations for this discrepancy, Räsänen et al. conclude that the apparent conundrum of limited gene flow but no obvious reproductive barriers could be very informative about the factors that constrain progress toward ecological speciation.

The second paper on three-spine stickleback, by D. I. Bolnick, considers the opposite conundrum: reproductive barriers are seemingly present but gene flow is not limited. Particularly, even though ecologically driven sympatric speciation does not always occur in sticklebacks, its theoretically necessary and sufficient conditions seem often to be present in nature. First, some populations experience strong competition for resources that causes extreme phenotypes to have higher fitness [28]. Second, assortative mating based on diet and morphology is present in some of these same lakes [29]. So how to solve this new conundrum? Using a simulation model, Bolnick demonstrates that the strengths of selection and assortative mating measured in lake populations in nature are too weak to cause sympatric speciation. Instead, lake stickleback appears to respond to disruptive selection through alternative means of reducing competition, such as increased genetic variance, sexual dimorphism, and phenotypic plasticity.

Another classic system for studying ecological speciation, or more generally adaptive radiation, is Anolis lizards of the Caribbean. In particular, many of the larger islands contain repeated radiations of similar “ecormorph” species in similar habitats [8]. Contrasting with this predictable and repeatable diversity on large islands, smaller islands contain only a few species. Y. Surget-Groba, H. Johansson, and R. S. Thorpe studied populations of Anolis roquet from Martinique. This species contains populations with divergent mitochondrial lineages, a consequence of previous allopatric episodes, and is distributed over a range of habitats. It can, therefore, be used to address the relative importance of past allopatry, present ecological differences, and their combination in determining progress toward ecological speciation. Using microsatellite markers, the authors find that geographic isolation alone does not result in significant population differentiation, habitat differences alone cause some differentiation, and geographic isolation plus habitat differences cause the strongest differentiation. The authors conclude that speciation is likely initiated in allopatry but is then completed following secondary contact only through the action of adaptation to different habitats.

Even though ecological differences are clearly important in the diversification of both plants and animals [1, 30], it remains uncertain as to whether the process is fundamentally the same or different between them. Part of the reason is that typical methods for studying ecological speciation differ between the two groups. In an effort to bridge this methodological divide, K. Ostevik, B. T. Moyers, G. L. Owens, and L. H. Rieseberg apply a common method of inference from animals to published studies on plants. In particular, ecological speciation is often inferred in animals based on evidence that independently derived populations show reproductive isolation if they come from different habitats but not if they come from similar habitats: that is, parallel speciation [31, 32]. Ostevik and coauthors review potential examples of ecological speciation in plants for evidence of parallel speciation. They find that very few plant systems provide such evidence, perhaps simply because not many studies have performed the necessary experiments. Alternatively, plants might differ fundamentally from animals in how ecological differences drive speciation, particularly due to the importance of behaviour in animals.

A current topic of interest in ecological speciation is whether strong selection acting on a single trait (strong selection or relatively weak selection acting on a greater number of traits (multifarious selection) is more common and more likely to complete the speciation process [11]. Using another well-studied model of ecological speciation, butterflies of the genus Lycaceides, C. F. Scholl, C. C. Nice, J. A. Fordyce, Z. Gompert, and M. L. Forister compared host-plant associated larval performance of butterflies from several populations of L. idas, L. melissa, and a species that originated through
hybridization between the two. By conducting a series of reciprocal rearing experiments, they found little to no evidence for local adaptation to the natal hosts. By putting these results into the context of the other previously studied ecological traits (e.g., host and mate preference, phenology, and egg adhesion [33, 34]), the authors constructed a schematic representation of the diversification within this butterfly species complex. They conclude that no single trait acts as a complete reproductive barrier between the three taxa and that most traits reduce gene flow only asymmetrically. The authors suggest the need for further study of multiple traits and reproductive barriers in other taxa.

4. Factors Affecting Progress toward Ecological Speciation

4.1. The Role of Pollinators/Parasites. In many cases of ecological speciation, we think of the populations in question colonizing and adapting to divergent environments/resources, such as different plants or other food types. However, environments can also “colonize” the populations in question that might then speciate as a result. Colonization by different pollinators and subsequent adaptation to them, for example, is expected to be particularly important for angiosperms. A particularly spectacular example involves sexually deceptive Orchids, where flowers mimic the scent and the appearance of female insects and are then pollinated during attempted copulation by males. In a review and meta-analysis of two Orchid genera, S. Xu, P. M. Schlüter, and F. P. Schildt find floral scent to be a key trait in both divergent selection and reproductive isolation. Other traits, including flower colour, morphology and phenology, also appear to play an important role in ecological speciation within this group. The authors also conclude that although sympatric speciation is likely rare in nature, it is particularly plausible in these Orchids.

Parasites can be thought of as another instance of different environments “colonizing” a focal species and then causing divergent/disruptive selection and (perhaps) ecological speciation. As outlined in the contribution by A. Karvonen and O. Seehausen, differences in parasites could contribute to ecological speciation in three major ways. First, divergent parasite communities could cause selection against locally adapted hosts that move between those communities, as well as any hybrids. Second, adaptation to divergent parasite communities could cause assortative mating to evolve as a pleiotropic by-product, such as through divergence in MHC genotypes that are under selection by parasites and also influence mate choice (see also [35]). Third, sexual selection might lead females in a given population to prefer males that are better adapted to local parasites and can thus achieve better condition. The authors conclude that although suggestive evidence exists for all three possibilities, more work is needed before the importance of parasites in ecological speciation can be confirmed.

4.2. The Role of Habitat Choice. The importance of habitat (or host) isolation in ecological speciation is widely recognized. This habitat isolation is determined by habitat choice (preference or avoidance), competition, and habitat performance (fitness differences between habitats) [36]. S. E. Webster, J. Galindo, J. W. Grahame, and R. K. Butlin propose a conceptual framework to study and classify traits involved in habitat choice, based on three largely independent criteria: (1) whether habitat choice allows the establishment of a stable polymorphism maintained by selection without interfering with mating randomness or if it also promotes assortative mating; (2) whether it involves one-allele or two-allele mechanisms of inheritance; (3) whether traits are of single or multiple effect [37], the latter when habitat choice is simultaneously under direct selection and contributes to assortative mating. The combination of these three criteria underlies ten different scenarios, which the authors visit using previously published empirical data. They argue that the speed and likelihood of ecological speciation depends on the mechanism of habitat choice and at which stage of the process it operates, with scenarios of one-allele and/or multiple-effect traits being more favorable. While these scenarios have rarely been distinguished in empirical studies, Webster et al. reason that such distinctions will help in the design of future studies and enable more informative comparisons among systems. In practice, however, the identification of the mechanisms involved and discriminating among different scenarios may sometimes be difficult, as exemplified by the case of the intertidal gastropod Littorina saxatilis, a model system for ecological speciation.

Hybrids resulting from the crosses between individuals from populations with different habitat preferences will tend to show interest in both parental habitats. This will increase gene flow between parental species, inhibiting reproductive isolation. Inspired by host-specific phytophagous insects, J. L. Feder, S. P. Egan, and A. A. Forbes ask, what if individuals choose their habitat based on avoidance rather than preference? According to the authors, hybrids for alleles involved in avoidance of alternate parental habitats may experience a kind of behavioral breakdown and accept none of the parental habitats, generating a postzygotic barrier to gene flow. Feder and collaborators determine the reasons why habitat avoidance is underappreciated in the study of ecological speciation (theoretical and empirical), and try to improve this issue. They propose new theoretical models and do not find strong theoretical impediments for habitat avoidance to evolve and generate hybrid behavioral inviability even for nonallopatric scenarios. They also suggest a physiological mechanism to explain how habitat specialists evolve to prefer a new habitat and avoid the original one. Feder et al. also document empirical support for this theory. Accumulated data on Rhagoletis pomonella and preliminary results on Utetes lectoides strongly suggest that avoidance has evolved in these species, contributing to postzygotic reproductive isolation. A literature survey in phytophagous insects reveals at least ten examples consistent with habitat avoidance, and three cases of behavior inviability in hybrids consistent with this mechanism. The authors also present suggestions and cautionary notes for design and interpretation of results when it comes to experiments on habitat choice.
Hybrid zones are particularly useful systems for determining whether differences in habitat preference or habitat-associated adaptation contribute to reproductive isolation. M. D. Carling and H. A. Thomassen investigate the effect of environmental variation on admixture in a hybrid zone between the Lazuli Bunting (Passerian amoena) and the Indigo Bunting (P. cyanea). They find that differences in environment explain interpopulation differences in the frequency and genetic composition of hybrids. This is not the first study to document an effect of environmental variation on the production or persistence of hybrids [38, 39] but Carling and Thomassen were also able to associate this pattern with specific environmental variables, particularly rainfall during the warmest months of the year. They discuss possible, complementary mechanistic explanations for these patterns, including habitat avoidance or preference in hybrids and habitat-dependent fitness. Their results indicate that inherent (i.e., non-geographic) barriers to gene flow between P. amoena and P. cyanea are environment dependent, which means these barriers could be ephemeral and vary in space and time.

S. P. Egan, G. R. Hood and J. R. Ott present one of the first direct tests of the role of habitat (host) isolation driven by host choice. Different populations of the gall wasp Belonocnema treatae feed on different oak species. Egan et al. first confirmed that B. treatae prefer their native host plant, with a stronger preference for females. They then demonstrated assortative mating among host populations, which was enhanced by the presence of the respective host plants. This enhancement was due to the fact that females usually mate on their host and that males also prefer their natal host plant. Therefore, host preference is directly responsible for reproductive isolation in B. treatae, by decreasing the probability of encounter between individuals from different host populations. The mechanism revealed here likely applies to many host/phytophagous or host/parasite systems.

4.3. The Role of Phenotypic Plasticity. Phenotypic plasticity, the ability of a single genotype to express different phenotypes under different environmental conditions, has long been seen as an alternative to genetic divergence, and therefore as potential constraint on adaptive evolution [40, 41]. More recently, however, adaptive phenotypic plasticity has been rehabilitated as a factor potentially favoring divergent evolution by enabling colonizing new niches, where divergent selection can then act on standing genetic variation [42]. B. M. Fitzpatrick reviews the possible effects of phenotypic plasticity on the two components of ecological speciation: local adaptation and reproductive isolation. He finds that both adaptive and maladaptive plasticity can promote or constrain ecological speciation, depending on several factors, and concludes that many aspects of how phenotypic plasticity acts have been underappreciated.

Several other papers in the special issue also provide potential examples of the role of plasticity in ecological speciation. For instance, N. V. Vallin and A. Qvarnström studied habitat choice in two hybridizing species of flycatchers. When the two species occur in sympatry, pied flycatchers are displaced from their preferred habitat due to competition with the dominant collared flycatchers. Cross-fostering experiments showed that rearing environment matters to recruits’ habitat choice more than does the environment of the genetic parents: pied flycatcher fledglings whose parents were displaced to pine habitats were more likely to return to nest in pine habitats. Thus, competition-mediated switches between habitats can cause a change of habitat choice through learning, which might then enhance reproductive isolation via ecological segregation. This role of plasticity and learning in habitat choice is also acknowledged in the contribution of Webster and collaborators.

5. Unanswered Questions and Future Directions

Although it is widely recognized that ecological speciation can occur without gene flow between diverging groups of individuals [43], the recognition of its importance has grown because of recent evidence for speciation with gene flow [44]. If gene flow commonly occurs during divergence, some mechanism, such as divergent selection must also occur frequently to counteract the homogenizing effect of gene flow. The manuscripts in this special issue, and a plethora of other recent publications [45–50], have made great strides in advancing our understanding of ecological speciation. These allow us to identify several key factors that affect progress toward ecological speciation, such as habitat choice (preference and avoidance), phenotypic plasticity, role of pollinators/parasites, complex biological interactions such as facilitation, as well as geographical context. However, for most cases, our understanding is still incomplete. For instance, the circumstances under which plasticity favors or inhibits adaptation, mate choice, and consequently ecological speciation are still largely unknown. Further insights will certainly arise from a multitude of empirical and theoretical studies, but certain areas of research are particularly likely to yield important results. For example, whereas we can rarely observe the time course of speciation in a single species, we can learn about factors affecting progress toward ecological speciation by studying and contrasting pairs of related populations at different points along the speciation continuum. Similarly, the study of parallel speciation may be highly informative. Such studies exist (e.g., [25, 26, 51, 52]), but we need many more systems where we can examine variation in progress toward ecological speciation. It is important that we also investigate instances where speciation fails, as these cases will advance our understanding of factors that constrain and enhance progress toward speciation. Furthermore, recent advances in DNA sequencing and statistical analysis offer an unprecedented opportunity to study the genetic basis and evolution of reproductive isolation during ecological speciation. The application of these new methods and models to ecologically well-studied systems have been and will be particularly informative [53, 54]. Finally, more studies using experimental manipulations to study the effects of key parameters on ecological speciation are badly needed, especially if they can be combined with an understanding of natural populations.


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References


Review Article

The Role of Parasitism in Adaptive Radiations—When Might Parasites Promote and When Might They Constrain Ecological Speciation?

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Research on speciation and adaptive radiation has flourished during the past decades, yet factors underlying initiation of reproductive isolation often remain unknown. Parasites represent important selective agents and have received renewed attention in speciation research. We review the literature on parasite-mediated divergent selection in context of ecological speciation and present empirical evidence for three nonexclusive mechanisms by which parasites might facilitate speciation: reduced viability or fecundity of immigrants and hybrids, assortative mating as a pleiotropic by-product of host adaptation, and ecologically-based sexual selection. We emphasise the lack of research on speciation continuums, which is why no study has yet made a convincing case for parasite driven divergent evolution to initiate the emergence of reproductive isolation. We also point interest towards selection imposed by single versus multiple parasite species, conceptually linking this to strength and multifariousness of selection. Moreover, we discuss how parasites, by manipulating behaviour or impairing sensory abilities of hosts, may change the form of selection that underlies speciation. We conclude that future studies should consider host populations at variable stages of the speciation process, and explore recurrent patterns of parasitism and resistance that could pinpoint the role of parasites in imposing the divergent selection that initiates ecological speciation.

1. Introduction

Since the publication of the Darwin’s “Origin of species” one and a half centuries ago, processes and mechanisms by which new species arise have fascinated evolutionary biologists. It is increasingly apparent that the rich biodiversity found on our planet has, at least partly, evolved in bursts of adaptive diversification, associated with the quick origin of new species, referred to as adaptive radiation [1, 2]. The intensive research on speciation of the past 20+ years, initiated perhaps by the publication of “Speciation and its consequences” [3], has produced much support for the hypothesis of speciation through divergent natural selection, often referred to as “ecological speciation” [4–8]. Ecological speciation research has now begun to integrate ecological and genomic research towards the identification of genes that are important at the onset of ecological speciation in a few systems [9–13]. However, at the same time, some of the most basic questions, such as what factors initiate and drive the emergence of reproductive isolation between diverging populations, remain unanswered for all but a handful of systems. Traditionally, research on ecological speciation has focused on habitat and trophic specialization and on the role of resource competition, as drivers of divergence and reproductive isolation within and between populations [14, 15]. Some of the recent empirical evidence
supports the role of these mechanisms (reviewed in [6, 8]). Moreover, predation has classically been considered as an important potential driver of divergence [16], and this idea has recently been explored in a number of papers (e.g., [17, 18]).

Parasitism is a predominant biological interaction in the wild [19, 20], but it has received relatively little attention in speciation research. Parasites live on the expense of other organisms by taking some or all of the energy they need from their host. Because of this peculiar life style, parasites have significant ecological and evolutionary consequences for hosts and host populations [21–24]. Potentially, infections might also initiate, facilitate, or reinforce speciation by imposing selective pressures that differ in form and strength from those imposed by the abiotic environment. Parasites may also impose a range of interrelated effects on host appearance, behaviour, condition, and, importantly, defence system. Classical papers have identified parasites as important sources of divergent selection [25, 26] and there is strong evidence to support their role as mediators of species coexistence [27, 28]. However, while this has led some authors to make far-reaching statements about the role of parasites in driving host diversification, evidence for speciation driven by parasites is very limited (though evidence may be strong for intraspecific genetic host diversity). The reasons for this lack of evidence are several: most studies to date are correlational and cannot separate cause and effect regarding diversity in parasites and hosts. For example, while some studies conclude that parasite diversity is a result of host diversity (e.g., [29, 30]), others have concluded the opposite even with the same data sets [31]. Coevolution that commonly prevails in host-parasite interactions is predicted to generate diversity at least in some constellations [32], and there is wide-spread empirical support for parasites diverging in response to host speciation ([33–35], [36] for a model). Such speciation may be ecological but is mediated by resource specialization and not by parasites. Yet, in cases of cospeciation, it can be difficult to interpret which one (if any) of the coevolving partners actually triggered the speciation in the other one. Moreover, divergence in parasite infections is commonly associated with divergence in food regimes and habitat [37–39]. This makes it difficult to infer parasite-mediated host divergence when there is coincident multivariate divergent selection between niches. It is also possible, and supported by some data, that parasites may actually prevent host speciation [40, 41].

In the present paper, we review and discuss the role that parasites might have in ecological speciation and adaptive radiation of their hosts. We go through the existing literature on the theory of parasite-mediated selection and discuss mechanisms that could lead to reproductive isolation in allopatric, parapatric or sympatric host populations, and the prerequisites for these mechanisms to operate. We then review the empirical literature on parasite-mediated speciation with an emphasis on fishes and birds. Hoping not to miss recent publications, we viewed all papers published in the past two years (May 2009 to July 2011) that were retrieved from Web of Science using the combination of search terms “parasite” and “speciation.” We also point out some important tests on the theory of parasite-mediated ecological speciation which are currently lacking. Essentially, these concern the initial stages of the speciation process, that is, at which stage of the speciation continuum do parasite infections become divergent among the host populations, and do they importantly restrict the gene flow between host populations? Also, we contrast the role of diversity of a parasite community with the role of single parasite species in driving parasite-mediated speciation, conceptually linking this to discussion on multifariousness of selection and the strength of selection. Finally, we discuss how different types of infections that, for example, alter host behaviour or visual abilities, could influence the process of speciation, or its reversal. We limit this review to metazoan and microparasite (protozoans, bacteria, and viruses) infections, while acknowledging that reproductive isolation and speciation may occur also in other fascinating parasitic interactions. These include, for example, brood parasitism in birds [42], where the interaction differs from “traditional” host-parasite systems as the parasite is not physically attached to the host, and symbiotic bacteria-host interactions, where mating preference can develop as a side effect of host adaptation to the environment [43]. We also restrict our review of empirical evidence to the zoological literature, but acknowledge that there is a larger body of evidence for speciation in plants driven by coevolution with pathogens and predators (see [33] as a classical starting point). There is also a wealth of recent literature on speciation in microbial systems, such as bacteria-phage interactions (e.g., [44, 45]), which is not considered here. We provide examples mainly from fishes and birds where some of the best case studies of ecological speciation and adaptive radiation exist and significant progress has been made in testing predictions from models of parasite-mediated speciation.

2. Prerequisites for Parasite-Mediated Divergent Selection

There are three main prerequisites for parasite-mediated divergent selection to operate in natural host populations. First and the most obvious is that infections should differ within or between the host populations. This can happen in allopatric host populations experiencing differences in diversity or magnitude of infections, but also in sympatric or parapatric populations where heterogeneities in ecological (the extent of exposure) or genetic (susceptibility) predisposition to infection create subgroups or subpopulations that have different infection levels. Overall, heterogeneities in infections within a host species inhabiting different geographical areas represent one of the best known phenomena in host-parasite interactions, and basically lay the foundations for investigating parasite-mediated divergent selection. For example, it is well known that ecological factors such as differences in host population structure or in environmental factors may generate variation in infection among populations of one host species (e.g., [46–48]). Typically, this is seen as a decrease in similarity of parasite species composition with increasing geographical distance among the host populations [49, 50] or even among different
locations within one host population [51]. Overall, such heterogeneities of infections could generate highly variable conditions for parasite-mediated selection.

The second prerequisite for parasite-mediated divergent selection is that differences in infections should remain reasonably constant among the host populations through time, thus maintaining the direction and perhaps also the strength of the divergent selection. For example, infections could be highly predictable with the same species composition and more or less similar infection intensities occurring in hosts every year, or show high levels of stochastic year-to-year variation among the host populations causing parasite-mediated selection to fluctuate in strength and direction and making consistent divergent selection unlikely. Similarly, spatial repeatability of infections across replicated host populations can be important when evaluating the role of parasites in speciation. In particular, such repeatability could reveal patterns of parallel ecological speciation, which is discussed in more detail below. Moreover, if host divergence is more likely across populations when certain parasite species are present (or absent), this can support the role of these parasites in host divergence. We come back also to this topic later in this paper.

The third important prerequisite for parasite-mediated divergent selection is that infections impose fitness consequences for the hosts and that these are sufficiently strong to overrule possible conflicting fitness consequences of other factors. This is required for parasites to actually impose net divergent selection between host populations. Such fitness consequences are generally assumed because parasites take the energy they need from the hosts which may result in reduced host condition and reproduction. Testing it, however, requires empirical measurement of fitness in nature or in reciprocal transplants that simulate natural conditions, whereas measurement of infection-related fitness components is insufficient. An important feature of host-parasite interactions is that wild hosts are typically infected with a range of parasite species at the same time. For example, in aquatic systems, individual fish hosts are commonly infected with dozens of parasite species simultaneously (e.g., [46, 47]). This is important in terms of direction and magnitude of selection. Under such circumstances, parasite-mediated divergent selection could be driven by a single parasite species having major impact on host viability or reproduction. Alternatively, selection could represent joint effects of multiple parasite species, each with unique types of effects on the host and possibly opposing effects in terms of divergent selection (e.g., see recent discussion in Eizaguirre and Lenz [52] on selection on MHC polymorphism). Separating such effects in natural host populations is a demanding task, which is discussed more below.

3. Mechanisms and Empirical Evidence of Parasite-Mediated Host Speciation

In a review on this subject eight years ago, Summers et al. [53] concluded that theory suggests that parasite-host coevolution might enhance speciation rates in both parasites and hosts, but empirical evidence for it was lacking. Since then, new empirical evidence has been gathered, and some of it supports the hypothesis of parasite-mediated ecological speciation, yet overall the empirical support is still scant. Some of the best data to test the hypothesis come from freshwater fish and from birds. Progress has recently been made in some of these key systems in identifying differences in infections among populations, ecotypes and/or sister species (the first prerequisite for parasite-mediated selection), and connecting these to possible mechanisms initiating, facilitating, or maintaining host population divergence and speciation. Table 1 summarizes some of the best studied examples. Here, we first review the existing literature on divergent parasite faunas in ecotypes of freshwater fishes where much new data have been gathered recently. Second, we bring up examples of studies that have gone further into testing predictions of mechanisms of parasite-mediated speciation and discuss these under the three categories of mechanisms: reduced immigrant and hybrid viability or fecundity, pleiotropy, and ecologically based sexual selection. For this second part of the review, we do not restrict ourselves to fish.

3.1. Divergent Parasite Infections. Despite the wealth of the literature on heterogeneities in parasite infections across host species and populations, surprisingly few empirical studies have investigated differences in parasite species composition in sympatric and parapatric host ecotypes or sister species in the context of parasite-mediated divergent selection and speciation. In fishes, such systems include salmonid and three-spine stickleback populations in the northern hemisphere, as well as cichlid fishes in East African great lakes (Table 1). For example, parapatric lake and river populations of sticklebacks in northern Germany differ in parasite species composition so that lake populations harbour a significantly higher diversity of infections [54–56]. Differences in parasitism have also been reported between marine and freshwater ecotypes of stickleback [57], as well as between sympatric stickleback species specializing on benthic and limnetic environments in lakes of Western Canada [58]. In all of these systems, divergent patterns of infection are most likely explained by differences in parasite transmission between different environments or by adaptation of the immune defence to these habitats [54, 56]. Other systems in the northern hemisphere also include whitefish and Arctic charr in lakes in Norway, where ecotypes and species inhabiting pelagic versus benthic habitats, and profundal versus benthic/pelagic habitats, respectively, show significant differences in parasite infections [59, 60]. Similar differences in infections have also been reported from four ecotypes of Arctic charr in a large lake in Iceland (Figure 1); ecotypes inhabiting littoral areas are more heavily infected with parasites transmitted through snails while the pelagic ecotypes harbour higher numbers of cestode infections transmitted trophically through copepods [61]. Moreover, we have recently observed differences in parasitism between whitefish populations and species reproducing at different depths in Swiss prealpine lakes [62].
Divergent parasite infections have also been described from cichlid fish in the lakes of East Africa, especially Lake Malawi and Lake Victoria. These systems are particular as they harbour a tremendous diversity of hundreds of cichlid fish species each that have emerged in the lakes in a few ten thousand to one or two million years [63–65], representing spectacular examples both of biodiversity and adaptive radiation, and of the high rates with which these can emerge. Recently, Maan et al. [66] described divergent parasite species composition in the closely related sister species *Pundamilia pundamilia* and *P. nyererei* of Lake Victoria. These differences were caused mainly by larval nematodes in the internal organs and ectoparasitic copepods associated with feeding more benthically in shallower water or more limnetically and slightly deeper. Similarly, heterogeneous infections have been reported in Lake Malawi, where the closely related species *Pseudotropheus fainzilberi* and *P. emmiltos* show divergent parasite species composition particularly in terms of certain ectoparasitic and endoparasitic infections [67].

Overall, such differences in infections fulfil the first prerequisite of parasite-mediated divergent selection and support the idea of a possible role of parasites in ecological speciation. However, it is still difficult to evaluate the generality of these findings. This is first because the number of empirical studies describing divergent parasitism among host ecotypes is still quite limited and examples only come from few relatively well-known systems. Second, it is possible that there is an ascertainment bias in the literature so that studies reporting nonsignificant differences in infections tend to not get published. This would be particularly likely with hosts in early stages of the speciation continuum if infections are not yet significantly divergent. However, we point to the necessity of such data in detail below. Overall, differences in parasite infections between diverging hosts alone do not reveal mechanisms underlying speciation, which we will discuss next.

### 3.2. Mechanisms of Parasite-Mediated Host Speciation

Speciation is a complex process, typically characterized by simultaneous operation of several factors and a cascade of events from initiation to completion. One of the most challenging problems in speciation research is to determine the relative importance to initiating, stabilizing, and completing the process of the many factors that typically vary between populations, incipient and sister species. Given that speciation is most readily defined as the evolutionary emergence of intrinsic reproductive barriers between populations, the most central question in speciation research is which factors drive its emergence, and what is the sequence in which they typically play? In this paper we are concerned with the mechanisms by which parasites could initiate the emergence of reproductive isolation, or facilitate or reinforce it after it had been initiated by other (ecological) factors. This also leads to a key question: at which stage of the speciation process do infections become divergent and begin to reduce gene flow between the host populations? In other words, do host divergence and the initiation of reproductive isolation follow divergence in parasite infections, or vice versa?

We consider three nonexclusive categories of mechanisms (Figure 2).

1. **Direct natural selection**: reproductive isolation due to parasite-mediated reduction of immigrant and hybrid viability or fecundity [68].
2. **Pleiotropy**: direct natural selection operates on the genes of the immune system, and the latter pleiotropically affect mate choice [69].
3. **Ecologically based sexual selection**: reproductive isolation due to parasite-mediated divergent sexual selection [8].

The first two categories of mechanisms could be considered byproduct speciation mechanisms, although the first one in particular may require reinforcement selection for completion of speciation. The third mechanism could be considered reinforcement-like speciation [8].

#### 3.2.1. Tests of Parasite-Mediated Viability or Fecundity Loss in Immigrants and Hybrids

In theory, adaptation to habitat-specific parasite challenges in ecotypes experiencing divergent parasite infections could facilitate reproductive isolation between the ecotypes through parasite-mediated selection against immigrants that acquire higher infection load outside their habitat, or hybrids that show nonoptimal resistance against the parasites and higher infection in either habitat (Figure 2). Selection against immigrants was recently investigated in marine and freshwater sticklebacks in Scotland and in Canada [70]. In these systems, anadromous marine fish, ancestral populations to the freshwater ecotypes, regularly migrate to freshwater to breed, but are still reproductively isolated from the resident, sympatric freshwater ecotypes. Using transplant experiments of lab-raised fish to simulate dispersal and antihelminthic treatment, MacColl and Chapman [70] demonstrated that ancestral-type marine sticklebacks contract higher burdens of novel parasites when introduced to freshwater, than in saltwater and suffer a growth cost as a direct result. Susceptibility to parasites and their detrimental effect in freshwater was less in derived, freshwater fish from evolutionarily young populations, possibly as a result of selection for resistance. MacColl and Chapman [70] concluded that differences in infections could impose selection against migrants from the sea into freshwater populations, but they did not test for selection against migrants in the opposite direction. Similar evidence comes from mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*), a passerine bird where immigrant males were more heavily infected with bloodborne *Haemoproteus* parasites and had lower mating success [71]. The authors suggested that immigrant birds may be immunologically disadvantaged, possibly due to a lack of previous experience with the local parasite fauna, resulting in low mating success.

A related mechanism by which direct natural selection could act in generating reproductive isolation is reduced fitness in hybrids, that is, offspring of two divergently adapted individuals from environments or habitats that differ in parasite infections suffer reduced viability or fecundity, for example, because their intermediate resistance
profiles do not match with either of the environments. There is a wealth of empirical literature on parasitism in animal species hybrids, and a small number of studies report higher infection rates of hybrid individuals, reviewed by Fritz et al. [72] and Moulia [73]. However, most of these studies deal with only distantly related species and interpretation in the context of speciation is problematic. In the context of the present paper, we are interested in examples involving ecotypes, sibling species, or young sister species. We go through recent examples from sticklebacks, mountain white-crowned sparrows and other birds, all of which actually speak against the hypothesis of parasite-driven hybrid inviability and infecundity. Rauch et al. [55] studied hybrids between stickleback from lake populations harbouring high parasite infections and river populations with fewer infections in Northern Germany. Hybrids with intermediate defence profiles in terms of MHC did not suffer higher parasite infections in reciprocal infection trials in either lake or river environments. Similar evidence against the hypothesis of parasite-mediated selection against hybrids has also been presented from collared and pied flycatchers, where individuals living in the hybrid zone of these two sister species showed intermediate prevalence of *Haemoproteus* blood parasites, as well as intermediate immune responses to infection [74] (Figure 3).

Even stronger evidence against the hypothesis of parasite-mediated selection against hybrids comes from work on the mountain white-crowned sparrows. Studying an outbred population for which it was known that parasites reduce fitness, MacDougall-Shackleton et al. [75] found that haematozoan parasite load was significantly negatively correlated with two complementary measures of microsatellite variability. The authors suggested that heterozygote advantage in terms of parasite load may counteract the high parasitism of immigrants (see above), who are likely to produce the most heterozygous offspring (Figure 3). A similar situation has also been reported in a population of song sparrows (*Melospiza melodia*), a species in which females often display strong preferences for local male song, and that is thought to undergo speciation in parts of its range [76]. Here too immigrants were less likely than residents to breed, but the outbred offspring of these immigrants had higher survivorship [77]. Perhaps the best evidence for parasite-induced loss of hybrid viability comes from studies on hybrid zones between eastern and western house mice [72, 73]. This is considered as classical tension zone where allopatric lineages with well-divergent genomes meet and hybridize such that some hybrid genotypes suffer intrinsic incompatibilities. House mice F1 hybrids enjoy reduced parasite susceptibility but hybrid breakdown is apparent in higher generation hybrids.

Overall, it is difficult to draw general conclusions on the role of parasite-induced hybrid inviability or infecundity in speciation processes as evidence for parasite-mediated
It is important to note that coevolution in host-parasite interactions may either facilitate hybridisation and gene flow or isolation and speciation, depending on the dynamics of coevolution (reviewed in [53]). For example, locally adapted parasites should have higher success in their resident hosts, providing an advantage to immigrants and hybrids in the hosts, whose genetic profile cannot be matched by the locally adapted parasites (i.e., the enemy release hypothesis in invasion biology). On the other hand, if local hosts are well adapted to their local parasites, and parasites are consequently not locally adapted, resident hosts should have equal or higher resistance than immigrants and hybrids. In theory, the situation where parasites are ahead of their hosts, should favour speciation between parasite populations but constrain speciation between host populations, whereas the reverse should facilitate speciation between host populations [53]. Few empirical studies of parasite-mediated speciation have explicitly looked at this.

We also point out that the above coevolutionary scenarios between parasites and hosts could commence not only at the level of different parasite species compositions, but also at parasite genotype compositions. Under such circumstances, different coevolutionary dynamics driving divergent parasite-mediated selection between different environments could take place with seemingly identical parasite species assemblies that are “cryptically divergent” showing different genotype composition between the environments. Conceptually, this can be seen as an extension to the hypothesis on divergent selection between contrasting environments.

Moreover, it is important to note that host-parasite interactions commonly show high levels of genetic polymorphism that could fuel speciation potential in parasites and/or hosts. In general, such variation could be maintained by different combinations of genotype by environment interactions (G × G, G × E or G × G × E) [79, 80], for example, as a consequence of parasite-parasite interactions within a coinfecting parasite community [81], or because of effects of environment on host susceptibility [82]. However, genetic polymorphism does not necessarily lead to emergence of new species if factors mediating divergent selection between host populations are absent.

3.2.2. Tests of Pleiotropy: MHC and Mate Choice. Reproductive isolation can also emerge as a byproduct of parasite-mediated divergent evolution at the genes of the immune system that pleiotropically affect mate choice (Figure 2). This includes the highly polymorphic family of genes in the major histocompatibility complex (MHC) that encode antigen-presenting molecules and have an important role in identification of non-self-particles and activation of adaptive immunity. They are also often involved in mate choice [69], thus having a pleiotropic role in parasite resistance and reproductive behaviour. The role of MHC in immune defence and mate choice has recently been reviewed by Eizaguirre and Lenz [52]. In theory, MHC-mediated mate choice may lead to assortative mating in host populations, albeit under restricted conditions [83]. This has recently received empirical support in some systems [54] (Figure 4).
Figure 3: Top left: in the hybrid zone between pied flycatcher (*Ficedula hypoleuca*) and collared flycatcher (*Ficedula albicollis*) hybrids showed intermediate prevalence of *Haemoproteus* blood parasites compared to the parental species, and also intermediate immune responses to infection [74] (photo shows a male hybrid *F. hypoleuca × F. albicollis*, courtesy of Miroslav Král). Bottom left: haematozoan blood parasites can be agents of severe selection in birds (photo shows *Haemoproteus multipigmentatus* infecting red blood cells of endemic Galápagos doves [84], courtesy of Gediminas Valkiunas). Right panels: in the mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) heterozygote advantage in terms of reduced parasite load may counteract elevated parasitism of immigrants, who are likely to produce the most heterozygous offspring [75] (photo courtesy of Bob Steele). Figure reproduced with permission of the Royal Society of London from MacDougall-Shackleton et al. [75].

Figure 4: Top left: a nuptial male of a lake ecotype of the Three-spined stickleback (*Gasterosteus aculeatus* species complex, photo Ole Seehausen). Bottom left: a breeding male stickleback and a series of *Schistocephalus* cestodes that were found in its body cavity (photo courtesy of Kay Lucek). These tapeworms change the behaviour of stickleback and effectively castrate them. Right: mean number of ectoparasitic *Gyrodactylus* monogenean parasites (log transformed) on Schleswig Holstein lake (grey bars) and river (white bars) sticklebacks, with or without an MHC haplotype G. Occurrence of the haplotype coincides with higher resistance against the parasite in the river ecotype while there is a tendency for the opposite pattern in the lake ecotype. Figure produced with permission from data in Eizaguirre et al. [54].
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<td>Fish</td>
<td>Three-spine stickleback sympatric species pairs in Vancouver lakes (Canada)</td>
<td>Reproductively isolated species</td>
<td>Yes in Paxton lake, no in Priest lake</td>
<td>Yes; limnetic species have much more cestodes, fewer mollusks and different trematodes</td>
<td>Divergence in MHC profiles</td>
<td>Not known</td>
<td>No data</td>
<td>No data</td>
<td>Yes</td>
<td>Yes, the limnetic species display redder male breeding dress and have lower MHC allele diversity</td>
<td>Yes, it is at least partly based on male breeding dress</td>
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<td>Three-spine stickleback marine versus freshwater populations on Vancouver coast (Canada)</td>
<td>Parapatric populations, perhaps species</td>
<td>No data, but very likely</td>
<td>Not known (but likely between the Sea and freshwaters)</td>
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<td>Freshwater residents are less susceptible than marine counterparts to infestation with freshwater parasites; the reverse is not known to freshwater residents</td>
<td>Not known</td>
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<td>Three-spine stickleback marine versus freshwater species in Scotland</td>
<td>Parapatric populations, perhaps species</td>
<td>No data, but very likely</td>
<td>Yes, freshwater residents are heavily infested by a cestode</td>
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<td>Three-spine stickleback parapatric lake and river populations in Schleswig Holstein (Germany)</td>
<td>Parapatric populations, perhaps quite old</td>
<td>Lake and stream populations are no direct sister taxon but belong to geographically more widespread reciprocally monophyletic clades</td>
<td>Yes, lake populations harbor larger numbers of parasites compared to river populations</td>
<td>Divergence in MHC profiles, immunological parameters and habitat specific resistance</td>
<td>Not known, but unlikely given the wide geographical distribution of the lake and the stream clade</td>
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<tr>
<td>Three-spine stickleback, marine versus freshwater populations in Atlantic Canada</td>
<td>Parapatric populations</td>
<td>No data</td>
<td>Yes, different parasite taxa are abundant in different environments</td>
<td>Not known</td>
<td>No data</td>
<td>Yes</td>
<td>Weakly so, and labile to environmental conditions</td>
<td>Parapatric</td>
<td>[57]</td>
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<tr>
<td>Lake Victoria cichlids <em>Pundamilia pundamilia</em> and <em>Pundamilia nyererei</em></td>
<td>Complete speciation continuum, but parasites only studied at the complete speciation stage</td>
<td>Yes, from microsatellite and AFLP data</td>
<td>Not known but perhaps not likely given that other traits diverge very early in the process</td>
<td>Not known</td>
<td>No data</td>
<td>Yes</td>
<td>Female preference for male nuptial coloration, which appears to be a revealing signal at least in one of the species</td>
<td>Geographically sympatric, ecologically parapatric</td>
<td>[10, 66, 101], Selz et al. Manuscript</td>
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<td>Lake Malawi cichlids <em>Pseudotropheus fainzilberi</em> and <em>P. emmiltos</em></td>
<td>Reproductively isolated species with perhaps mild gene flow</td>
<td>Probably not direct sister species</td>
<td>Yes</td>
<td>Different MHC profiles between the populations</td>
<td>No data</td>
<td>No data</td>
<td>Yes</td>
<td>Divergent MHC profiles between the populations</td>
<td>Female preference for male nuptial coloration, which appears to be a revealing signal at least in one of the species</td>
<td>Geographically parapatric</td>
<td>[67, 143, 144]</td>
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<tr>
<td>Alpine charr in Norway</td>
<td>Conspecific parapatric populations</td>
<td>No data</td>
<td>Yes, profundal ecotype harbours significantly fewer infections compared to littoral/pelagic ecotype</td>
<td>No data</td>
<td>Not known</td>
<td>Yes</td>
<td>Not known</td>
<td>No tested</td>
<td>Parapatric</td>
<td>[59]</td>
<td></td>
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<tr>
<td>Benthic and pelagic ecotypes of whitefish in two lakes in northern Norway</td>
<td>Conspecific parapatric populations</td>
<td>No data</td>
<td>Yes, divergence in infections corresponds with the divergence in diet of the ecotypes</td>
<td>No data</td>
<td>Not known</td>
<td>Yes</td>
<td>Not known</td>
<td>No tested</td>
<td>Parapatric</td>
<td>[60]</td>
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<td><strong>Populations of guppies in Trinidad</strong></td>
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<tr>
<td>Allopatric/parapatric populations from different rivers with no reproductive isolation</td>
<td>No data</td>
<td>Yes, populations were differently infected with <em>Gyrodactylus monogenea</em></td>
<td>Yes, different parasite taxa</td>
<td>No data</td>
<td>Not known</td>
<td>No data</td>
<td>Not data</td>
<td>Not known</td>
<td>Yes, different MHC allele frequencies between the populations</td>
<td>Not tested</td>
<td>Yes, different MHC allele frequencies between the populations</td>
<td>MHC could act as a homogenizing mechanism counteracting speciation among the populations although mechanisms is not tested</td>
</tr>
<tr>
<td><strong>Whitefish in Swiss prealpine lakes</strong></td>
<td>Complete speciation (or speciation reversal) continuum</td>
<td>Yes, from microsatellite and AFLP data</td>
<td>Yes, different parasite taxa</td>
<td>No data</td>
<td>Not known</td>
<td>No data</td>
<td>No data</td>
<td>Not known</td>
<td>No data</td>
<td>Not known</td>
<td>Not known</td>
<td>Geographically sympatric, ecologically parapatric</td>
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<tr>
<td><strong>Bird</strong></td>
<td>Mountain white-crowned sparrows</td>
<td>Conspecific populations</td>
<td>No data, but very likely</td>
<td>No data</td>
<td>No data</td>
<td>No known</td>
<td>Not known</td>
<td>Not known</td>
<td>Yes, immigrant individuals had higher infection rates and lower reproductive success</td>
<td>No, hybrids were at a selective advantage</td>
<td>Yes, partially</td>
<td>Not known, but resident males sing a local dialect</td>
</tr>
<tr>
<td><strong>Bird</strong></td>
<td>Collared and pied flycatchers</td>
<td>Distinct sympatric species with a hybrid zone</td>
<td>Probably, recently diverged species</td>
<td>Differences in infection rate between the species</td>
<td>No difference in immune responses between the species</td>
<td>Not known</td>
<td>No data</td>
<td>Yes</td>
<td>No data</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Parapatric</td>
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**Table 1:** Continued.
On the one hand, MHC-mediated mate selection is known to often favour outbreeding and associated disassortative mating between and within host populations [69, 85]. MHC disassortative mating preferences can in principle increase fitness of choosy parents because a disproportionate number of offspring would be high fitness MHC heterozygotes [86]. There is also evidence to suggest that intermediate MHC diversity results in higher fitness for an individual since low diversity allows some parasites to escape immune detection and very high diversity increases the risk of autoimmunity [87]. Under such circumstances, divergent optimality of MHC allele frequencies in contrasting environments that differ in parasite exposure may lead to or facilitate ecological speciation through mate choice for well-adapted MHC profiles in each environment [88, 89]. For example, experimental work with stickleback suggests that female sticklebacks use evolutionarily conserved structural features of MHC peptide ligands to evaluate MHC diversity of their prospective mating partners [88]. It should be noted here that MHC-mediated divergent sexual selection does not require individuals to be infected with the parasite species that has driven the evolutionary divergence in MHC profiles, or with any other parasite. This is in contrast to situations of direct natural selection, for example, when the fitness of immigrants or hybrids is reduced by the actual infection (see above). However, suboptimal or superoptimal MHC profiles of hybrids could still cause higher parasite infection and reduced fitness, adding to reproductive isolation through viability selection as suggested by theoretical models [89].

Much of the empirical work on the interactions between parasitism and diversity of MHC genes has been conducted in contrasting infection environments. Recent progress has been made especially in ecotype and species pairs of three-spine sticklebacks in northern Germany and Canada. Lake and river ecotypes of sticklebacks in Germany harbour significantly different parasite communities so that the lake populations are infected with a higher diversity of parasite species [54, 90]. This difference is known to be linked positively with the diversity of MHC genes; more heavily infected lake populations show higher diversity of MHC compared to river populations [54]. Moreover, this is accompanied by variation in resistance between ecotypes where the less-infected river ecotype shows reduced immunocompetence [56]. Such between-habitat variation in the pools of MHC alleles suggests operation of parasite-mediated selection, although it does neither imply divergent selection, nor exclude simultaneous action of genetic drift [54]. Somewhat contrasting results on MHC diversity in sticklebacks, however, come from Canadian benthic-limnetic species pairs. In this system, the limnetic ecotype carries higher number of parasites, especially those species likely to impose selection, than the benthic ecotype [58], but still harbour fewer MHC alleles [91].

Outside the stickleback systems, evidence for divergent MHC profiles in ecotypes or young sister species is scarce. One of the few fish examples is the study by Blais et al. on sympatric cichlid species in Lake Malawi [67]. These authors demonstrated high polymorphism in MHC, divergence in...
MHC allele frequencies, and differences in parasite infections between these closely related fish species, supporting the idea of parasite-mediated divergent selection. We expect that more tests will be conducted in ecotypes and young sister species of fish, and other host taxa in the near future. This will be important in establishing the generality of relationships and unravelling the somewhat contradictory results obtained in different systems even with the same host taxon.

The above examples demonstrate heterogeneous MHC profiles between ecotypes and young species. However, very few studies have taken the necessary next step towards testing the MHC-pleiotropy speciation hypothesis by looking into MHC-mediated assortative mating among ecotypes. Again, experimental work here includes that in two stickleback systems. Mate choice trials by Eizaguirre et al. [92] in seminatural enclosures revealed that female sticklebacks from lake populations in northern Germany showed preferences for males with an intermediate MHC diversity, and for males carrying an MHC haplotype that provides protection against a locally common parasite. Subsequently, the same authors extended this approach to lake and river ecotypes and found, using a flow channel design, that females preferred the odour of their sympatric males [54]. They concluded from these studies that parasite-induced divergent selection on MHC diversity and for local adaptation could act as a mechanism of speciation through the pleiotropic role of MHC in mate choice. However, actual assortative mating between the lake and stream populations remains to be demonstrated. Other authors found in common garden experiments that assortative mating between lake and stream sticklebacks may often not evolve despite divergent selection on ecological traits [93; Räsänen et al. this volume [94]). However, it is nevertheless possible that such assortative mating occurs in nature owing to environmental influences.

Assortative mating mediated by MHC has also been studied in saltwater versus freshwater sticklebacks in the St. Lawrence River in Canada [57], where the populations differed significantly in the frequency of MHC alleles and in the communities of helminth parasites. Strong signatures of natural selection on MHC genes were inferred in the freshwater, but not in the marine population. Relationships between parasite load and MHC diversity were indicative of balancing selection, but only within the freshwater population. The latter result is in accordance with other studies on sticklebacks suggesting maximisation of host fitness at intermediate rather than maximal MHC diversity in some environments [95, 96]. Mating trials found signals of MHC-mediated mate choice to be weak and significantly influenced by environmental conditions (salinity; [57]). By allowing full mating contact to the fish, these authors demonstrated differences between the ecotypes in the importance of MHC-mediated mate choice, and very strong environment dependence where mating preferences with regard to MHC were sometimes inversed depending on whether fish were tested in their own or a different salinity environment. The authors concluded that MHC probably plays an important role when individuals evaluate prospective mates, but that MHC-mediated mate choice decisions depend on the environmental conditions and are not necessarily underlying the propensity towards assortative mating [57].

Evidence for a counteracting role of MHC in host speciation, on the other hand, comes from Trinidad guppies [40]. These authors found significantly lower divergence in MHC among guppy populations than expected from divergence at neutral loci and concluded that stabilizing selection on MHC and its pleiotropic role in mate choice could act as a homogenizing mechanism among the populations [40]. Evidence for stabilizing selection on some and divergent selection on other MHC loci was observed in Alpine trout populations adapting to steep thermal gradients [97]. It is clear from these contrasting results that more examples from different systems are needed to address the generality of parasite-mediated divergent selection on MHC and the role of MHC cues in mate choice among diverging host populations.

3.2.3. Tests of Parasite-Mediated Divergent Sexual Selection. Reproductive isolation in this scenario emerges by sexual selection for direct benefits (i.e., healthy mates) or for heritable fitness (i.e., parasite resistance) as an indirect consequence of adaptation to different parasite challenges (Figure 2). In theory, divergent sexual selection is an effective mechanism of reproductive isolation [98], while parasites are considered mediators of mate choice [99]. Several aspects of parasitism may lead to population divergence under these circumstances (discussed recently in [8]). For example, infections are commonly related to habitat and diet, and they impose selection on signal design, maintain genetic variation and honesty of sexual signalling through the cost they impose on signal production and maintenance, as well as have health consequences for their hosts which may filter down to mate attraction and selection [8]. There are several alternative scenarios for how this may lead to population divergence, but most of the studies so far have been conducted on interactions between parasitism and mating preferences. These are related to the seminal paper by Hamilton and Zuk [99] on the tradeoffs between individuals’ ability to resist parasite infections and produce extravagant sexual ornamentation by which sexual selection for healthier mates ensures heritable resistance to offspring. Such selection could also promote speciation if ecological factors that lead to divergent parasite infections result in reproductive isolation through selection for parasite resistance [8].

Empirical evidence comes from freshwater fishes. For example, Skarstein et al. [100] showed that individual Arctic char (Salvelinus alpinus) within one population had marked differences in habitat and diet, and this correlated also with parasite infection and breeding colouration. Such variation could facilitate niche-specific adaptation in hosts and set the initial stages for speciation through divergent sexual selection. Similarly, in cichlid fish, males with bright red (Pundamilia nyererei) or bright blue (P. pundamilia) colouration were found to carry lower parasite infections than males with duller coloration [66, 101]. At the same time, females use male nuptial colouration in intra- and interspecific mate choice [102, 103] (Figure 5). However, what role
the divergent parasite infections have in speciation in this system is unclear because parasites have been investigated only in the well-advanced stage of speciation, and because the differences in infections coincide with differentiation in diet, microhabitat, and visual system [66], which supports the idea that parasite-mediated divergent selection is often just one component of multifarious divergent selection between habitats [104]. Overall, it remains to be tested in all of the systems if the preference of females for more resistant males actually results in production of offspring that are more resistant to parasites found in each particular environment [8].

4. Future Directions: Missing Tests of Parasite-Mediated Divergent Selection

The empirical examples reviewed above demonstrate recent progress in identifying differences in parasite infections between host populations that occupy contrasting environments and have diverged in phenotypic and genetic traits, and in linking these differences to assortative mating and speciation (Table 1). However, it is also clear that many more empirical tests of the role of parasite-mediated divergent selection in these and other taxa are needed before the generality of some of the more trenchant findings can be assessed. Next we will discuss three categories of tests of parasite-mediated speciation that are currently lacking: parasite-mediated divergent selection along a speciation continuum, the strength of selection versus multifariousness of selection, and measuring the relative rates of adaptation in parasites and in hosts.

4.1. Divergent Selective Pressures along a Continuum of Speciation. We ought to understand at which point of the speciation process parasite assemblages become sufficiently divergent to reduce gene flow between host populations. This is particularly true because divergent infections are usually associated with divergent habitat and/or diet, and hence rarely come isolated from other sources of divergent selection. At present, it remains unknown whether divergence in parasitism could itself initiate the divergence of host populations, or rather follows the divergence of host populations initiated by other ecological factors. In the second case, it is unknown in most cases whether divergence in parasitism is consequential or inconsequential to speciation. Answering these questions requires approaches that capture the entire continuum of speciation (see [104, 105]). This should include replicated populations occupying contrasting habitats, but showing no apparent divergence, and extending to well-differentiated ecotypes and incipient species and eventually all the way to fully isolated sister species [104, 105]. However, all empirical investigations of parasite effects known to us have dealt each with just one stage in the continuum of the speciation process, and in fact often with species that are already divergent in many different traits (Table 1). Nevertheless, there are several potential systems where the speciation continuum approach could be applied. In fishes, the strongest candidate populations would most likely come from African and Central American great lake cichlid fish, and from sticklebacks, char, and whitefish species in postglacial lakes.

At the same time, studies should consider spatiotemporal variation, or consistency, in parasite-induced selective pressures. Investigating infections over replicated pairs of host populations could reveal if infection patterns are consistently different, for example, among hosts inhabiting two distinct environments. Under such circumstances, infections could drive parallel ecological speciation in different populations [106–108]. In reality, infections almost always differ to some extent among populations in terms of species composition and infection intensities because of heterogeneities in local conditions for parasite transmission. For example, associations between host diet and infection from trophically transmitted parasites could show habitat-specific variation. However, if the “core” of parasites (including one or several species) that underlies selective pressures and host divergence remains more or less the same, parallelism in host divergence among different populations could be observed. Approaches that capture parallelism along a continuum of host speciation could tackle not only the importance of infections in speciation process per se, but the significance of individual parasites species as well (see below).

Similarly, temporal stability of infections on the time scale of host generations would indicate if the strength of selection is stable enough to result in divergence of host populations. So far, the extent of spatiotemporal variation or consistency is unknown in most systems that include a limited number of host populations and/or a narrow temporal window for observations (but see Knudsen et al. [39] for long-term data on Arctic char in Norway). Attempts to relate such temporal patterns to variation in the progression of speciation have also been few [109]. In general, what is needed are long-term data on spatiotemporal consistency of divergent parasite infections in replicated pairs of host populations that are at different stages of speciation. This is a demanding task, but could help in answering fundamental questions such as whether speciation takes place only in populations where costly infections do not fluctuate randomly. Most importantly, patterns of parasitism must be investigated in an integrated speciation perspective alongside various other ecological and genetic factors some of which would typically interrelate with parasitism. This should include effective combinations of field surveys and experimental approaches to generate real insights into the question of how parasites affect the speciation process in relation to other factors initiating or promoting ecological speciation. For example, divergent parasitism may initiate speciation on its own right, or it may be a necessary force in the transition from early stages of divergent adaptation to assortatively mating species, but it may also latch onto other mechanisms of divergent evolution without being consequential for the process of speciation.

4.2. Strength versus Multifariousness of Parasite-Mediated Selection. Divergent selection may act on a single trait, a few traits or many traits at the same time, and in each of these cases selection may have a single or many sources. In
a recent review, Nosil et al. [5] related this to hypotheses about “stronger selection” when the completion of speciation depends on the strength of selection on a single trait, or “multifarious selection” when completion of speciation is more likely driven by independent selection on several traits [5]. A predominant feature of natural host-parasite interactions is that hosts harbour a community of parasite species that are interconnected through the use of the common host as a resource, interspecific parasite-parasite interactions, and direct and indirect effects of host immunity (e.g., [110, 111]). Parasite-mediated divergent selection can vary between host populations both in strength of selection imposed by one parasite or in the number of parasite species together exerting multifarious selection. However, strength and dimensionality of parasite-mediated selection in the context of host divergence and speciation is currently largely unknown. Empirical examples typically report multiple parasite species infections in the diverging host populations, some of which show differences between the populations. Hence, current data tend to suggest that diverging ecotypes are often exposed to divergent parasite species assemblages (Table 1). The role of individual species and especially their joint effects, however, are poorly known.

In sticklebacks, parasite diversity tends at least sometimes to be positively associated with host diversity at MHC loci [54, 112] suggesting that multiple parasite species could be driving host population divergence at multiple MHC loci. However, typically some parasite species impose stronger selection, for example, because they are more numerous, have larger body size, or infect a more vital organ of the host. For example in German sticklebacks, resistance against Gyrodactylus parasitises is determined by a certain MHC haplotype in river populations, whereas that same haplotype when occurring in the lake population results in slightly higher parasite numbers [54] (Figure 4). Given that MHC-mediated resistance correlates with reproductive success [92], the opposite outcomes of infection in different environments could facilitate speciation [54]. This supports the role of this individual parasite species in divergence of the host populations. However, variable outcomes of selection in different combinations of parasite species are nevertheless likely; one species could drive the selection on its own or in some populations favouring the idea of strong selection, while effects from multiple species predominate in other systems supporting multifarious selection. It is also generally unknown if selection imposed by different parasite species takes the same direction with regard to fitness effects of migration and gene flow, and hence speciation. For example, Keller et al. [97] found evidence for stabilizing selection on one and divergent selection of another locus in the MHC gene family in Alpine trouts. On the scale of entire parasite communities, such interactions may be complex and difficult to resolve. However, search for recurring patterns of infections and resistance in replicated pairs of similar ecotypes, or along a speciation continuum, could provide some tools to tackle the relative importance of strong and multifarious selection.

4.3. Measuring Relative Rates of Adaptation in Parasites and Hosts. Measuring relative rates of adaptation in parasites and their hosts could allow empirical testing of the theoretical predictions about when parasite-host coevolution should facilitate and when it should actually constrain speciation in host populations. Theoretical considerations predict that parasite-host coevolution should facilitate speciation in host populations when host populations can adapt to the parasite community that infects them. In this situation, gene flow from nonadapted host populations could be maladaptive, and assortative mating between host populations may evolve by either of the three mechanisms reviewed above. On the other hand, when parasites adapt to their local host population (reviews in [113–115]), gene flow into the host population from outside could be adaptive because it would provide genetic variants not known to the local parasites that escape parasitism. Parasite-host coevolution should constrain speciation in host populations in this scenario [53]. Given that most parasites have faster generation times than their hosts, it seems that the second scenario could apply quite often. Conditions favourable to host speciation would include situations where hosts are ahead of their parasites in the coevolutionary race, for example, because parasite adaptation is genetically constrained. They may also entail situations where host-parasite coevolution happens in one-to-many or many-to-many constellations. In such situations, adapted hosts may cope well with the local community of diverse parasites without coevolving closely with any one of them. Very few existing data speak to these theoretical predictions. One interesting corollary of the above is that the odds of speciation in hosts and those in parasites should often be negatively correlated. Very high speciation rates in parasites compared to their hosts have been described for example in monogenean platyhelminthes infecting freshwater fishes [116]. To the extent that these parasites evolve host-specific adaptations, they might facilitate outbreeding and constrain speciation in host populations.

5. Reversal of Speciation

Some of the best examples of adaptive radiation come from freshwater fishes, and in several of these the frequent reversal of speciation has also been described particularly as a consequence of human activity. The most compelling evidence comes from cichlid fishes in Lake Victoria, stickleback in Western Canada, and whitefish and ciscoes in central Europe and North America [117, 118]. Rapid adaptive radiation in Lake Victoria has produced a magnificent diversity of hundreds of cichlid species. One likely mechanism driving speciation involves evolution in the visual system and of visual signals in response to heterogeneous light conditions, and its effects on mate choice and speciation through mechanisms related to sensory drive [10]. However, increasing eutrophication of the lake from the 1920s and subsequent turbidity of the water have resulted in relaxation
of the diversity-maintaining mate selection and collapse of species diversity in some parts of the lake [119]. Similar collapses of species diversity through loss of reproductive isolation following anthropogenic impacts have occurred in ciscoes of the Laurentian Great lakes, in whitefish of Swiss prealpine lakes [118], and in Canadian sticklebacks [120].

5.1. Role for Parasites? The above observations relate to the hypothesis of parasite-mediated speciation in two ways. First, investigating mechanisms and processes of speciation reversal may also shed light onto those promoting ecological speciation. In general, reversal of speciation may (re)create a speciation continuum, where formerly distinct sympatric species become admixed to variable degrees, with variation in time, space, or both. Capturing and studying such changes as they happen in nature could provide effective tools to evaluate the possible role of parasites in divergence of natural host populations. For example, environmental changes such as pollution and eutrophication may affect not only the host community, but could change biomass and reduce the diversity of parasite species as well, with species having multiple host life cycles probably being among the most prone to extinction [47, 121]. Changes that relax parasite-mediated divergent selection among host populations could lead to gradual loss of reproductive isolation, but also reveal the role of specific parasite species that had maintained the divergence. Additionally, experimental work is also needed to address the possible role of parasites in breaking down speciation or preventing it, something that is known very little about.

Second, it is possible that certain parasite species may directly drive reversal or prevention of speciation. In systems where divergence of species is maintained by divergent adaptation in the visual system, and visually mediated sexual selection, such as African cichlids and sticklebacks, parasites that influence host vision might have effects comparable to loss of visibility due to eutrophication. One group of such parasites are those of the genus Diplomastomum that infect eyes of a range of freshwater fish species around the world causing cataracts and partial or total blindness (Figure 1; [122]). Infection has dramatic effects on fish [123–125] and it could also impair the ability of fish to visually select mates. For example, females with impaired visual ability could be less choosy as they cannot properly assess the quality of the male colouration or courtship. Impaired vision of males, on the other hand, could affect their ability to compete with other males, court females or, in case of sticklebacks, build nests. The risk of eye fluke infection is typically variable in both space and time, which can result even in total absence of infection from some lakes (Karvonen et al., unpublished). This could set up very different conditions for host divergence mediated by vision-based mate choice within allopatric host populations. For example, it is tempting to ask if divergence of the hosts under such circumstances would be more likely in populations that are only moderately, or not at all, infected with eye flukes, conceptually linking this to the effect of eutrophication on the loss of diversity of cichlid fishes in Lake Victoria [119]. Comparative experimental approaches could shed light onto these associations and will be needed to link the probability of infection with the degree of host population divergence observed in nature.

Also other infections could lead to alterations in mate choice and sexual selection, possibly resulting in reduced probability of host divergence or in reversal of speciation. For example, several parasite species alter the behaviour or appearance of their hosts as a side effect of infection or by actively manipulating the host to improve transmission [21, 23]. Depending on the prevalence of such infections in a host population, this could dramatically change sexual selection. For example, it has been suggested that effects of Schistocephalus infection on the growth of sticklebacks directly affect assortative mating which is based at least partly on size [126]. Coinfections with several parasite species that commonly prevail in wild hosts may also change circumstances for sexual selection in hosts. For example, whereas some parasite species use hosts as transmission vehicles to the next host by manipulating their behaviour, other species may use the same host individuals for reproduction and completion of the life cycle. Two parasite species with complex life cycles can also use the same host individual as a vehicle to different definitive host taxa, such as fish and bird. These situations can result in conflicts between the opposing parasite interests (e.g., [127, 128]), when the outcome for host behaviour and sexual selection may depend on which of the parasite species dominates the coinfection situation. The important point emerging from this is that the variety of interrelated mechanisms by which parasitic infections can reduce host fitness (e.g., depletion of energy, changes in appearance or behaviour, increase in susceptibility to predation) can set up very different conditions for sexual selection depending on how common different types of (co)infections are.

5.2. Speciation by Hybridization. Hybridisation between species can also give rise to new species [129–131]. Hybrid speciation is particularly likely when the hybrid population is able to colonize a niche that is not occupied by either parental species and makes it spatially isolated from both the parental species [132, 133]. A scenario that would be relevant in the context of our review is if hybrids are able to colonize a novel niche where infections differ from those in the parental niches. In principle it is then even possible that parasite-mediated reversal of speciation, happening in parts of the larger range of the parental species, could lead to the local emergence of a new hybrid species. There currently is a growing number of empirical examples of hybrid speciation both from plant and animal systems (reviewed in [131, 134]), and it will be interesting to see if future studies will pick up a signature of parasite-mediated selection in some of these cases.

5.3. Parasitism, Phenotypic Plasticity, and Speciation. The above scenarios of infections that influence the condition or behaviour of their host may also be relevant in the context of old and recent discussions on the role of plasticity in generating reproductive isolation that precedes any adaptive divergence (e.g., [135, 136]; Fitzpatrick, this issue [137]). In host-parasite interactions, such plasticity could be observed,
for example, if reduction or change in host condition or behaviour as a result of infection leads to condition-dependent habitat choice and thus promotes reproductive isolation between individuals (see [18]). Phenotypic variation in individual’s ability to avoid infections by shifting to another habitat away from the infection source (e.g., [139]) could also contribute to segregation. Moreover, such behaviours could be further shaped or reinforced by the earlier infection experience and “learning” of an individual [140, 141]. Overall, there are several ways how plastic responses of hosts to a parasite infection could contribute to reproductive isolation, but they wait for empirical tests.

6. Conclusions
Empirical evidence available today has just begun to unravel mechanisms of parasite-mediated selection and how these affect the course of ecological speciation. Evidence for direct natural selection is equivocal because reduced viability or fecundity of immigrants may be compensated by hybrid vigour. Evidence for pleiotropy through effects of MHC on assortative mating is mixed too because divergent and stabilizing selection both occur, sometimes even in the same populations, and because MHC may not be an overriding mate choice cue. Evidence for parasite-mediated divergent sexual selection is scarce and incomplete, with best examples perhaps from Arctic charr and cichlid fish. The future should see more detailed investigations of parasitism and host resistance at all stages of speciation. Importantly, such investigations must become part and parcel of an integrated analytical and experimental research framework on ecological speciation. Particular emphasis should be placed on studying the entire speciation continuum and not just the beginning and end. This is the only way to determine whether, how and at what stage parasites begin to influence a divergence process that actually has ecological speciation as its end product. Further, empirical testing of the theoretical predictions about when parasite-host coevolution should facilitate and when it should constrain speciation in host populations has great potential to make major contributions to an integration of the currently still disparate literature. Clearly expectations should depend on the relative rates of and constrains to evolutionary adaptation in parasites and their hosts, and it is likely that the current lack of data on this explains some of the variable and contradictory results of empirical studies. Finally, many more speciating taxa ought to be studied to identify generalities.

References


Review Article

Parallel Ecological Speciation in Plants?

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Populations that have independently evolved reproductive isolation from their ancestors while remaining reproductively cohesive have undergone parallel speciation. A specific type of parallel speciation, known as parallel ecological speciation, is one of several forms of evidence for ecology’s role in speciation. In this paper we search the literature for candidate examples of parallel ecological speciation in plants. We use four explicit criteria (independence, isolation, compatibility, and selection) to judge the strength of evidence for each potential case. We find that evidence for parallel ecological speciation in plants is unexpectedly scarce, especially relative to the many well-characterized systems in animals. This does not imply that ecological speciation is uncommon in plants. It only implies that evidence from parallel ecological speciation is rare. Potential explanations for the lack of convincing examples include a lack of rigorous testing and the possibility that plants are less prone to parallel ecological speciation than animals.

1. Introduction

The past two decades have witnessed a dramatic shift in studies of speciation from an emphasis on stochastic and other nonecological processes to a focus on ecological mechanisms of speciation. Indeed, the proverbial pendulum has swung so far toward ecology that some authors have argued that essentially all plausible types of speciation involve ecological processes [1]. Despite the pervasive role of natural selection in evolution, evidence that ecologically based divergent natural selection is the primary cause of reproductive isolation (ecological speciation sensu Schluter, 2001 [2]) is often weak or incomplete in case studies [3].

To more critically evaluate the importance of ecological speciation in nature, several authors have suggested methods for reliably inferring ecological speciation [2–5]. These include (1) direct measurements of divergent ecological selection on parental genotypes (e.g., immigrant inviability) or hybrids (i.e., extrinsic postzygotic isolation) in the different environments; (2) natural selection studies showing that phenotypic differences underlying premating reproductive barriers are a consequence of divergent ecological selection; (3) molecular marker studies of selection against immigrants (i.e., isolation by adaptation); (4) molecular evolutionary studies linking intrinsic genetic incompatibilities with divergent ecological selection; and (5) tests of parallel ecological speciation, which is the process in which related lineages independently evolve similar traits that confer shared reproductive isolation from their ancestral populations [6].

In plants, widespread application of the first two methods listed above points to an important role for ecology in speciation. For example, there is a long tradition of reciprocal transplant studies since early in the 20th century, and there is abundant evidence of immigrant inviability among recently diverged populations or species [7, 8]. Because habitats often are spatially segregated, divergent habitat adaptation results in ecogeographic isolation, which is considered by many botanists to be the most important reproductive barrier in plants [1, 9]. Likewise, studies that examine the relative importance of different components of reproductive isolation in plants indicate that ecologically based reproductive barriers often play a key role in the early stages of plant speciation [8]. However, evidence of extrinsic postzygotic isolation in plants is surprisingly weak, possibly because of heterosis [8, 10]. Also, few studies have explicitly tested for isolation by adaptation in plants [1] or for ecological causes of hybrid incompatibilities (reviewed in [11]). The evidence for parallel ecological speciation is perhaps least
clear because while “recurrent” formation of plant species and races is thought to be common [12], the evidence underlying these apparent examples has not been examined systematically.

On the other hand, parallel speciation is regularly cited as evidence for ecological speciation in animals (e.g., [6, 13–22]), and the evidence for many individual cases of parallel ecological speciation is strong. For example, the threespine stickleback has undergone several well-documented parallel transitions between environments. The most well-known case is likely the independent origin of “benthic” and “limnetic” ecotypes in at least five British Columbian lakes [23, 24]. Another well-studied system is the marine snail *Littorina saxatilis*, which has repeatedly evolved pairs of ecotypes on the rocky coasts of Northwestern Europe [25]. Numerous other strong candidates for parallel ecological speciation are found in animals, including but not limited to lake whitefish [26], cave fish [27], walking sticks [20], scincid lizards [21], lamprey [28], electric fish [29], horseshoe bats [30], and possibly even in the genetic model organism *Drosophila melanogaster* [31], though not all of these examples are fully validated with the criteria described below.

Here we use explicit criteria to evaluate the strength of evidence for parallel ecological speciation in plants. We evaluate plant systems using criteria that are more often used to evaluate animal systems because comparable evaluations across taxa are important for determining general patterns of speciation. We find that evidence for parallel ecological speciation in plants is surprisingly rare in comparison to animals and provide potential explanations for this finding.

### 2. Studying Parallel Ecological Speciation

Parallel speciation is the process in which related lineages independently evolve similar traits that confer shared reproductive isolation from their ancestral populations [6]. It is good evidence that selection drove the evolution of reproductive isolation, as it is unlikely that the same barriers would arise independently by chance [6]. Schluter and Nagel [6] listed three criteria for parallel speciation: (1) related lineages that make up the new descendent populations are phylogenetically independent; (2) descendent populations are reproductively isolated from ancestral populations; (3) independently derived descendent populations are not reproductively isolated from each other. They add that an adaptive mechanism must be identified to show that natural selection drove the evolution of reproductive isolation [6]. Together, these four conditions are the evidence necessary to demonstrate the process we refer to as parallel ecological speciation. We choose to use the term “parallel” over the alternative term “convergent” because of the initial similarity of the independent lineages [32–34] and because this vocabulary is consistent with the original description of the process [6].

If we apply these criteria to well-known examples of parallel or “recurrent” speciation in plants [12], it is clear that botanists and zoologists are mainly studying different things. For example, many auto- and allopolyploid species have multiple independent origins (reviewed in [35]), in which independently derived polyploid lineages are reproductively isolated from their common ancestor but not from one another. Additionally, a high proportion of homoploid hybrid species studied arose in parallel [36]. Although there is evidence that natural selection is important in polyploid and hybrid speciation [10, 37, 38], the genomic changes that accompany polyploidization and hybridization reduce our ability to show that parallel ecological selection was the primary driver of reproductive isolation. This differentiates parallel polyploid and hybrid speciation from parallel ecological speciation described above and represented in the animal cases listed previously.

In cases of parallel ecological speciation, the independent descendent populations are found in a new environment where they experience new and shared ecological selection that causes speciation. However, not all cases in which multiple transitions to a new environment are associated with repeated speciation events represent true parallel ecological speciation. Several possible patterns exist and are shown in Figure 1. In parallel ecological speciation, ancestral and descendent groups each represent single compatible groups (Figure 1(a)). However, one can also envision several other patterns, in which either the ancestral or descendent groups (or both) represent multiple compatibility groups (Figures 1(b)–1(d)).

The pattern in which the descendent groups are incompatible with one another (Figure 1(b)) can be caused by mutation-order speciation in which the same selective pressure leads to different genetic changes in the multiple populations [4]. The isolation between ancestral groups could also be the result of drift. The third pattern (Figure 1(c)) has been called “replicated ecological speciation” [39] and is made up of multiple distinct speciation events. Studying similarities and differences between these replicate speciation events can help identify general patterns of speciation [39]. Finally, the last pattern (Figure 1(d)) is to our knowledge novel, and we do not know of any empirical examples.

In this survey, however, we are only interested in parallel ecological speciation (Figure 1(a)), which tells us something more specific than other patterns. In particular, parallel ecological speciation indicates that all of the new barriers present are predominantly if not entirely due to natural selection, whereas in the other cases, other forces may have been at play along with natural selection. For example, if the descendent populations are reproductively isolated from one another (Figures 1(b) and 1(c)), it is plausible that changes, driven by processes other than ecological adaptation, caused the isolation between descendent populations as well as the isolation across habitats. This means that testing the compatibility of descendent populations is essential for documenting parallel ecological speciation. However, this test is not necessary for demonstrating all forms of evidence for ecological speciation.

When studying parallel ecological speciation, it is also useful to recognize that evidence of parallelism may or may not extend across multiple levels of biological organization. Although the individuals of the descendent species must have
the same isolating trait, this common trait is not necessarily governed by the same mutation, gene, or even pathway in the different replicates. Even when the same mutations are responsible for parallel transitions in a given isolating trait, these mutations can be either the result of recurrent \textit{de novo} mutation or standing variation. While independent genetic changes simplify the task of reconstructing population and trait histories, parallel changes from standing genetic variation can be useful for pinpointing regions of the genome responsible for ecological selection and reproductive isolation [40].

### 3. Literature Survey

For this survey, we searched the scientific literature for evidence of parallel ecological speciation in plants. We used combinations of the words “parallel”, “recurrent”, “multiple”, “convergent” and “speciation”, “evolution”, “origins”, “reproductive barriers/isolation” as well as “paraphyly” as search terms in Web of Science and Google Scholar. We also examined all papers citing candidate examples and searched for additional papers about the candidate species. Levin [12] reviewed a number of potential cases of parallel ecological speciation in plants. Although his paper was not strictly about parallel ecological speciation, we consider all of his ecological examples in our table in order to revisit their validity and discuss new evidence for each case. We used explicit criteria to determine the strength of evidence for parallel ecological speciation. Specifically, we judged the strength of evidence for each of the aforementioned four criteria: independence, isolation, compatibility, and selection. To merit inclusion, we required that each newly identified case has at minimum weak direct evidence for repeated adaptation to similar environments (independence) or multiple origins of an isolating trait (isolation) and indirect evidence for the other condition. Evidence that an example failed to meet any of the four criteria resulted in its exclusion. As a result, several promising systems were not included in our table or appendix (e.g., \textit{Frankenia ericifolia} [41] and \textit{Heteropappus hispidus} ssp. \textit{Leptocladus} [42]). This was because \textit{Frankenia ericifolia} has only indirect evidence of both independence and isolation and \textit{Heteropappus hispidus} ssp. \textit{Leptocladus} has no evidence of isolating traits. Lastly, we evaluated a few of the frequently cited examples of parallel ecological speciation in animals for comparison. The list of
animal examples is not exhaustive and does not necessarily include all of the best cases.

The following sections discuss each criterion and the types and strengths of evidence we considered.

3.1. Independence. Parallel speciation requires that replicate lineages be phylogenetically independent, so that the “shared traits responsible for reproductive isolation evolved separately” [6]. Estimating phylogenetic independence can be difficult for recently diverged taxa because of inadequate resolution, hybridization and introgression, and deep coalescence. These issues are exacerbated when phylogenies are based on a small number of loci or if the loci employed have little phylogenetic information content (e.g., isozymes). Additionally, it is not strictly necessary for populations to be phylogenetically independent for reproductive barriers to evolve separately, if different genes, mutations, or genetic pathways are exploited by selection in populations that remain connected by gene flow.

Therefore, in this survey, strong evidence for independent evolution includes: (1) phylogenetic analyses supporting independence with multiple, phylogenetically informative loci or (2) direct evidence that a shared isolating trait has evolved independently (e.g., the trait is a result of different mutations in different populations). Phylogenetic analyses with a single locus, or with loci having little information content, are deemed weak direct evidence. Note that we consider genetic information from completely linked markers (e.g., multiple genes sequenced from the chloroplast genome) as information from one locus. Finally, indirect evidence for independence could include the improbability of long-distance colonization or gene flow between geographically separated but ecologically similar habitats, or phenotypes that are similar but not identical suggesting different genetic bases.

3.2. Isolation. As with any test of incipient or recent speciation, reproductive isolation must have evolved between descendant and ancestral populations, though not necessarily to completion. For strong evidence of isolation, we require experimental evidence for strong reproductive barriers that are genetically based, such as substantial differences in flowering time or pollination syndrome in a common garden and/or F1 hybrid inviability or sterility. Experimentally demonstrated weak but statistically significant reproductive barriers between diverging populations (including selection against immigrants and hybrids), or genetic divergence between locally diverging populations despite the opportunity for gene flow, are considered weak evidence for isolation. We view systems with apparent immigrant or hybrid inviability (e.g., serpentine adaptation), long-term persistence of divergent populations in sympatry, or strong divergence in mating system as indirect evidence that barriers likely exist. Although we would not consider isolation that has no genetic basis as evidence for parallel ecological speciation, we acknowledge that phenotypic plasticity can facilitate or impede the evolution of reproductive barriers and is an important consideration for studies of ecological speciation [43] (e.g., [44]). Furthermore, because explicitly testing that isolation is genetically based was rare in our candidate studies, we only required the genetic basis of isolation to be confirmed for cases to have strong direct evidence.

3.3. Compatibility. As we briefly discuss above, the lack of reproductive barriers between descendant populations is a key criterion distinguishing true parallel ecological speciation from other forms of replicated ecological speciation (Figure 1). For strong evidence of reproductive compatibility we require that descendant populations show little or no barriers to reproduction when experimentally crossed and minimal ecological divergence as demonstrated by reciprocal transplant or manipulative ecological experiments. If only one of these two components of compatibility were demonstrated, we consider the evidence to be weak. We also consider substantial genetic analysis showing little genetic differentiation at neutral markers between the descendant populations to be weak direct evidence for compatibility, although this might weaken the case for the criterion of independence. Lastly, if the evolution of environmental specificity or mating system is such that descendant populations are indistinguishable in these parallel traits and have no other phenotypic differences, we consider this indirect evidence for compatibility.

It is difficult to ascertain the strength of evidence for isolation and compatibility when multiple independent origins of self-fertilization (autogamy) have occurred. This is because replicate populations of selfers are likely to be as strongly isolated from one another as they are from the ancestral outcrossing populations. For this reason, replicated selfing lineages were excluded from consideration unless accompanied by the evolution of other reproductive barriers.

3.4. Selection. Without evidence of selection, parallel speciation cases can tell us nothing about ecology’s role in speciation. Because of this, we searched for evidence of the adaptive mechanism(s) underlying parallel isolation. For strong evidence, we include reciprocal transplants showing strong local adaptation, manipulative experiments relating adaptive traits to extrinsic fitness, and/or signatures of selective sweeps at loci underlying putatively adaptive traits. Similarly, reciprocal transplants showing weak local adaptation, manipulative experiments showing a weak relationship between traits and extrinsic fitness, or common garden experiments comparing $Q_{ST}$ to $F_{ST}$ are deemed weak evidence. Finally, we consider correlations between novel traits and environments or habitats to be indirect evidence for the role of selection.

4. Results of the Survey

The most striking result of this survey is that very few plant cases have strong evidence for two or more criteria of parallel ecological speciation, and most have only weak or indirect evidence for any of the criteria (Table 1; Appendix A). Only 3 of the 15 examples discussed by Levin [12] meet our
minimum requirements for inclusion in the table. We did identify 8 new candidate systems. These were Cerastium alpinum, Eucalyptus globulus, Geonoma macrostachys, Hieracium umbellatum var. vespertina, Lasthenia californica, Petunia axillaris, Schizanthus grahamii, and Streptanthus glandulosus. Some of the new candidate systems are quite promising, but none have strong evidence for all criteria. These cases and a review of Levin’s [12] examples are summarized in Table 1 and Appendix A. The animal cases used as a comparison are summarized in Table 2 and Appendix B. Although few of the animal cases have strong evidence for all the criteria either, these well-studied animal systems are more strongly supported than the plant examples.

Many of the putative plant cases lack evidence of compatibility among independent parallel populations. In fact, none of the examples have strong evidence for compatibility. This is unfortunate given the effectiveness of this criterion for demonstrating that ecological selection was the main cause of reproductive isolation in these systems—the primary reason for studying parallel ecological speciation in the first place. Hopefully, future studies in these systems will test for evidence of compatibility between suspected examples of parallel species. On the other hand, the criterion with the most evidence is the independent evolution of lineages that appear to be diverging in parallel. This is unsurprising given the widespread application of molecular phylogenetic methods in plants.

One of the best candidates of parallel ecological speciation in plants to date is Eucalyptus globulus [75] (Appendix A). In this case, three populations of E. globulus that inhabit granite headlands have a dwarfed morphology. Data from several nuclear and chloroplast markers show that each of the dwarfed populations is more closely related to its nearest tall population than to other dwarfed populations. Furthermore, there are two lines of evidence for isolation. First, the dwarfed populations flower earlier than the tall populations. Second, there is no evidence of pollen flow from the tall populations to the dwarfed populations despite a thorough examination of variation at microsatellite loci. However, the genetic basis of isolation, compatibility among

<table>
<thead>
<tr>
<th>Species</th>
<th>Min. number of origins</th>
<th>Independence</th>
<th>Isolation</th>
<th>Compatibility</th>
<th>Selection</th>
<th>Major parallel trait(s)</th>
<th>References</th>
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<tr>
<td>Alopecurus myosuroides&lt;sup&gt;lx&lt;/sup&gt;</td>
<td>Many</td>
<td>**</td>
<td>NA</td>
<td>NA</td>
<td>***</td>
<td>Herbicide tolerance</td>
<td>[45–51]</td>
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<td>Agrostis capillaries&lt;sup&gt;lx&lt;/sup&gt;</td>
<td>Many</td>
<td>*</td>
<td>NA</td>
<td>NA</td>
<td>**</td>
<td>Edaphic tolerance</td>
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<tr>
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<td>NA</td>
<td>*</td>
<td>**</td>
<td>Edaphic tolerance</td>
<td>[55–57]</td>
<td></td>
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<tr>
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<td>*</td>
<td>**</td>
<td>NA</td>
<td>**</td>
<td>Edaphic tolerance</td>
<td>[58, 59]</td>
</tr>
<tr>
<td>Armeria maritima&lt;sup&gt;lx&lt;/sup&gt;</td>
<td>NA</td>
<td>***</td>
<td>†</td>
<td>*</td>
<td></td>
<td>Flower colour</td>
<td>[60–63]</td>
</tr>
<tr>
<td>Chaenactis spp.&lt;sup&gt;x&lt;/sup&gt;</td>
<td>2</td>
<td>***</td>
<td>†</td>
<td>**</td>
<td></td>
<td>Flower colour</td>
<td>[64, 65]</td>
</tr>
<tr>
<td>Cerastium alpinum</td>
<td>2</td>
<td>**</td>
<td>*</td>
<td>NA</td>
<td>***</td>
<td>Edaphic tolerance</td>
<td>[66]</td>
</tr>
<tr>
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<td>*</td>
<td>NA</td>
<td>NA</td>
<td>*</td>
<td>Herbicide Tolerance</td>
<td>[67–70]</td>
</tr>
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<td>**</td>
<td>NA</td>
<td>NA</td>
<td>**</td>
<td>Leaf morphology</td>
<td>[71, 72]</td>
</tr>
<tr>
<td>Deschampsia caespitosa&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2</td>
<td>**</td>
<td>*</td>
<td>*</td>
<td></td>
<td>Edaphic tolerance</td>
<td>[73, 74]</td>
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<td>**</td>
<td>*</td>
<td></td>
<td>Dwarfed morphology</td>
<td>[75]</td>
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<td>3</td>
<td>**</td>
<td>**</td>
<td>*</td>
<td></td>
<td>Habitat type; reproductive strategy</td>
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<td>***</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>Edaphic tolerance</td>
<td>[81–87]</td>
</tr>
<tr>
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<td>6</td>
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<td>—</td>
<td>Flower colour</td>
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<td>NA</td>
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<td>NA</td>
<td>Spike morphology</td>
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<td>Poa annua&lt;sup&gt;lx&lt;/sup&gt;</td>
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<td>*</td>
<td>—</td>
<td>*</td>
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<td>Pollination syndrome, self-compatibility</td>
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<td>NA</td>
<td>NA</td>
<td>—</td>
<td>Edaphic tolerance</td>
<td>[97, 98]</td>
</tr>
<tr>
<td>Silene vulgaris&lt;sup&gt;lx&lt;/sup&gt;</td>
<td>2</td>
<td>*</td>
<td>*</td>
<td>NA</td>
<td>***</td>
<td>Edaphic tolerance</td>
<td>[99–102]</td>
</tr>
<tr>
<td>Streptanthus glandulosus</td>
<td>Many</td>
<td>**</td>
<td>*</td>
<td>**</td>
<td></td>
<td>Edaphic tolerance</td>
<td>[103–107]</td>
</tr>
</tbody>
</table>

<sup>d</sup>: Levin example.
<sup>lx</sup>: Example that would not have been included in this table had they not been reviewed in Levin [12].

Table 1: Candidate examples of parallel ecological speciation in plants showing the strength of evidence for each case. The details of each example can be found in Appendix A. The strength of evidence for each case was coded as follows: ****: strong direct evidence, ****: weak direct evidence, *: indirect evidence, —: no evidence, †: evidence against, and NA: no data.
dwarfed populations, and the selective advantage of being dwarfed still need to be confirmed in this example.

Other promising cases include Lasthenia californica [81] (Appendix A) and Schizanthus grahamii [96] (Appendix A). Lasthenia californica has strong evidence for parallel evolution of two races that have different flavonoid pigments and are found in different edaphic environments using phylogenetic analyses of ribosomal and chloroplast sequence and allozyme variation [81–84]. Race A is more tolerant of Na⁺ and Mg²⁺ and race C flowers earlier and produced more seed heads under drought conditions [85, 86]. However, isolation and compatibility between and within the races need to be confirmed. Conversely, there are clear reproductive barriers between Schizanthus grahamii and Schizanthus hookeri as they have different primary pollinators and experimental interspecific crosses produced no seeds [96]. However, it is not certain that these barriers arose multiple times independently because only chloroplast sequence data has been analyzed.

There are a few commonalities among the candidate examples. In many of the candidate systems ancestral and descendent forms have evolved particular edaphic tolerances and/or specific changes in reproductive phenotype or mating system. Under divergent ecological selection, these traits are likely to make ecological speciation relatively easier because they can cause assortative mating as a byproduct of divergent selection.

### 5. Why Is Parallel Ecological Speciation Seemingly Rare in Plants?

#### 5.1. Lack of Data

The lack of strong examples of parallel ecological speciation in plants is probably because botanists typically do not do the necessary tests. Perhaps this is because botanists have never doubted the importance of ecology in speciation [1, 7, 9] or because other methods of inference have been successful. However, we believe that it is useful to examine parallel ecological speciation explicitly in plants given how fruitful such studies have been in animals. In the present paper, this has allowed us to not only identify the key information that is missing in most potential case studies of parallel ecological speciation in plants, but also to recommend the experimental tests that are likely to be most profitable. For example, immigrant inviability is likely an important barrier in cases of parallel ecological speciation in plants. Therefore, reciprocal transplants between the new habitat types (as a test of compatibility) and among sites in a single habitat type (as a test of isolation) are crucial. A second essential set of tests that should be conducted is crosses between ancestral and descendent populations and crosses among populations in each habitat. It is surprising that these data are lacking given that both an explicit framework for studying parallel ecological speciation [6] and a list of possible cases [12] have been available for many years.

Furthermore, the signature of parallel speciation is easily lost. For example, if gene flow occurs between independently derived populations, the signals of phylogenetic independence may be lost. Conversely, if descendent lineages are geographically isolated (i.e., allopatric) but otherwise reproductively compatible, they will likely eventually evolve reproductive isolation from one another even if they were not originally isolated. Thus, the window of time in which parallel speciation can be detected may be relatively narrow. Interestingly, some of the strongest animal cases (Littorina, threespine stickleback, and whitefish) are no more than 40,000 years old (postglacial) [23, 108], and some are thought to be as young as 10,000 years [19]. However, we

### Table 2: Examples of parallel ecological speciation in animals showing the strength of evidence for each case. This list is not exhaustive and instead is a selection of well-studied cases serving as a comparison to the plant examples. The strength of evidence for each case was coded as follows: ∗∗∗: strong direct evidence, ∗∗: weak direct evidence, ∗: indirect evidence, —: no evidence, †: evidence against, and NA: no data.

<table>
<thead>
<tr>
<th>Species</th>
<th>Min. number of origins</th>
<th>Independence</th>
<th>Isolation</th>
<th>Compatibility</th>
<th>Selection</th>
<th>Major parallel trait(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astyanax cave fishes</td>
<td>2</td>
<td>**</td>
<td></td>
<td>***</td>
<td>***</td>
<td>Pigmentation, eye functionality</td>
<td>[27]</td>
</tr>
<tr>
<td>Coregonus clupeaformis</td>
<td>6</td>
<td>**</td>
<td></td>
<td>***</td>
<td>***</td>
<td>Body size and shape, foraging niche</td>
<td>[13, 108–115]</td>
</tr>
<tr>
<td>Coregonus lavaretus</td>
<td>3</td>
<td>**</td>
<td></td>
<td>***</td>
<td></td>
<td>Body size and shape, foraging niche</td>
<td>[14, 115–117]</td>
</tr>
<tr>
<td>Gasterosteus aculeatus benthic/limnetic</td>
<td>5</td>
<td>**</td>
<td></td>
<td>***</td>
<td></td>
<td>Body size and shape, foraging niche</td>
<td>[3, 15, 16, 23, 24, 118–122]</td>
</tr>
<tr>
<td>Gasterosteus aculeatus lake/stream</td>
<td>6</td>
<td>**</td>
<td></td>
<td>***</td>
<td></td>
<td>Body size and shape, foraging niche</td>
<td>[3, 23, 123–130]</td>
</tr>
<tr>
<td>Gasterosteus aculeatus anadromous/stream resident</td>
<td>many</td>
<td>**</td>
<td></td>
<td>***</td>
<td></td>
<td>Body size and shape, life history</td>
<td>[3, 17, 23, 131–133]</td>
</tr>
<tr>
<td>Littorina saxatilis-Galician Spain</td>
<td>3</td>
<td>**</td>
<td></td>
<td>***</td>
<td>***</td>
<td>Shell size and shape, microhabitat adaptation</td>
<td>[18, 19, 134–141]</td>
</tr>
<tr>
<td>Timema cristinae</td>
<td>NA</td>
<td>**</td>
<td></td>
<td>***</td>
<td></td>
<td>Color pattern, host plant adaptation</td>
<td>[20, 142–144]</td>
</tr>
</tbody>
</table>
see no reason why this window would be narrower in plants than animals. Thus, while the narrow window of detectability may account for the overall paucity of convincing examples of parallel ecological speciation in either the plant or animal kingdoms, it cannot explain why there are fewer examples in plants than in animals.

5.2. Plants Are Different. It is also possible that parallel ecological speciation is truly rare in plants. Considerable work would need to be done to validate this conjecture. However, should this pattern exist, there are several potential explanations. First, it is possible that the types of habitat distributions that promote parallel speciation in animals are more rare for plant populations. Many but not all of the animal examples involve adaptation to systems such as lakes or streams which are common, offer geographical isolation, and provide relatively homogeneous ecological environments. Perhaps these kinds of ecological opportunities are less frequent for plants? We think this explanation unlikely given that patchy environments (especially edaphic environments) are common in terrestrial ecosystems and parallel adaptation into those habitats occurs frequently.

It is also possible that plants have certain characteristics that make parallel ecological speciation unlikely or lack characteristics that promote parallel ecological speciation. This potential difference between plants and animals may be in part because behavior is not particularly relevant to plants. Behavior, especially behaviorally based mate preference, may be an important component of parallel ecological speciation in animal systems (though pollinator behavior in flowering plants may act analogously). Perhaps plants have no trait equivalent to body size in animals, which can act as a “magic trait” [145] to serve in both assortative mating and ecological adaptation. However, flowering time could be such a trait, and there are many examples of flowering time changing in new edaphic environments (e.g., Lord Howe Island palms [146]). On the other hand, flowering time may be quite constrained because partitioning flowering time requires narrower windows of flowering, which can have strong negative fitness consequences. Other potential traits are floral morphology and edaphic tolerances. Floral morphology may adapt to attract different pollinators and, consequently, lead to pollinator isolation. Similarly, the evolution of edaphic tolerance often leads to selection against immigrants.

The lack of evidence for parallel ecological speciation in plants is a mystery that may represent a key to understanding how species arise in plants. If parallel ecological speciation is more common than our survey suggests, then we can bolster existing evidence that ecology plays an important role in plant speciation. On the other hand, if parallel ecological speciation is determined to be rare, we can conclude that speciation may be less repeatable and more complicated than sometimes believed. We do not intend to imply that ecological speciation does not happen in plants. In fact, we believe it to be common. However, evidence of parallel ecological speciation in plants is not yet as convincing as it is for animal examples. We hope our study will spur additional investigation of the promising systems identified here, as well as provide guidance regarding the kinds of studies that should be performed in each system.

Appendices

A. Descriptions of Potential Examples of Parallel Ecological Speciation in Plants

Note that superscript “L” (¹) indicates that the example was reviewed in Levin [12].

A.1. Alopecurus myosuroides⁰. The black-grass Alopecurus myosuroides is an agricultural weed that has evolved resistance to herbicides in many locations, possibly independently [45]. Independent evolution may be occurring even on very local scales: Cavan et al. [46] used microsatellite data to show that four patches of resistant black grass in two neighbouring fields were independently derived from nonresistant plants. Herbicide resistance occurs either through plant metabolism, often polygenic, or via mutant ACCase alleles, and seven mutant resistance alleles have been identified [47–49]. A study of herbicide resistance in populations across Europe concluded that the same mutant ACCase alleles have appeared repeatedly [50]. No work has been done on reproductive barriers between resistant and nonresistant plants, although AFLP analysis shows little differentiation between resistant and nonresistant populations [51]. An important consideration in this case is that selection is human mediated and therefore unlikely to remain constant long enough to allow for speciation.

A.2. Agrostis capillaris⁰. The corrosion of zinc-galvanized electricity pylons in South Wales has created repeated patches of zinc-contaminated soil that have been colonized by the grass Agrostis capillaries. Zinc tolerance levels for A. capillaries plants vary from low to high across multiple pylons [52]. Tolerance appears to be polygenic and dependent on standing genetic variation [53]. Jain and Bradshaw [54] determined that seed and pollen dispersal is limited beyond 5 m, suggesting that tolerance is evolving independently at each pylon, although the still relatively small distances between pylons (300 m) do not rule out occasional pylon to pylon gene flow. Further work should establish if the populations are truly independent and measure barriers to gene flow between tolerant and non tolerant neighbouring populations.

A.3. Agrostis stolonifera⁰. Metal refining in Prescot, UK, caused considerable copper contamination to surrounding soil, and the grass Agrostis stolonifera has since then colonized a number of contaminated sites. Older sites were found to have more complete ground cover and a greater proportion of resistant individuals [55], suggesting that the evolution of tolerance is ongoing at younger sites. Morphological and isozyme analyses suggest, counterintuitively, that there is a reduction in clone number in uncontaminated sites compared to contaminated ones. All sites are centered on a single copper refinery, so the independence of the sites
is questionable. *Agrostis stolonifera* has also evolved salt tolerance in multiple inland and coastal sites, possibly independently [56, 57]. The strength of evidence for independent or parallel evolution in either of these cases is quite weak.

A.4. *Agrostis tenuis*. Copper-tolerant populations of *Agrostis tenuis*, from the UK and Germany, were shown to have different responses to copper using regression analysis [58]. This variability indicates that multiple genes or alleles are responsible for copper tolerance in different populations, although crossing experiments could further support this inference. Other work has shown asymmetric gene flow between tolerant and nontolerant populations with moderate levels of copper tolerance found in sites downwind of colonized mine tailings [59]. Although adult plants from mine sites showed high degrees of tolerance, seeds were not tested, so it is unknown if gene flow from nontolerant populations is reduced pre- or postzygotically. The adaptation to copper-contaminated soil at multiple sites may be parallel, or may be the result of the transmission of tolerant genotypes between mines. Without stronger evidence for independence, this case is very weak.

A.5. *Armeria maritima*. Populations of *Armeria maritima* across Europe vary in metal tolerance: those living on metaliferous soil are tolerant while those on uncontaminated soil are not [60]. Isozyme and nuclear marker data suggest that tolerance has evolved multiple times independently [61–63]. Furthermore, this tolerance is maintained even in the face of substantial gene flow between neighbouring nontolerant populations, indirect evidence for the strength of selection on the metaliferous soil. This does not appear to represent parallel speciation, however, as pollen fertility and gene flow are not reduced between populations with different levels of tolerance [61].

A.6. *Cerastium alpinum*. Enzyme phenotypes suggest that northern Europe was colonized by two postglacial lineages of *Cerastium alpinum* [64]. The two lineages are found on both serpentine and nonserpentine soils but a principle components analysis of enzyme phenotype does not reveal any clustering by soil type, suggesting that serpentine tolerance evolved independently in each lineage [64]. Further experiments manipulating Ni and Mg concentrations show that serpentine populations have higher tolerance to Ni and Mg [65]. No barriers to reproduction have been documented in this system although selection against immigrants seems likely.

A.7. *Chaenactis spp.*. Three closely related species of pin-cushion are found in California: *Chaenactis glabriuscula*, *C. stevioides*, and *C. fremontii*. *C. glabriuscula* is found in mesic habitat, has yellow flowers, and has n = 6 chromosomes while both *C. stevioides* and *C. fremontii* are found in desert habitat, have white flowers, and have n = 5 chromosomes. Cytological analysis indicated that *C. stevioides* and *C. fremontii* arose from independent aneuploid reductions [66]. Frequent natural hybrids between the species indicate that gene flow is possible although it may be limited by differences in edaphic preference. Future work should use molecular tools to verify the cytological data and quantify gene flow between species.

A.8. *Chenopodium album*. The agricultural weed *Chenopodium album* has developed resistance to triazine herbicides in multiple locations [67]. Early work showed that different resistant populations have distinct isozyme patterns in France [68] with at least two resistant genotypes in Canada [69]. Although a single amino acid mutation in the *psbA* gene has been linked to herbicide resistance [70], further molecular analysis should be performed to establish if this mutation has evolved independently. No work has been done on gene flow between populations.

A.9. *Crepis tectorum*. The degree of leaf dissection, a trait with fitness consequences, varies among populations of *Crepis tectorum* [71]. In the Baltic region, populations vary in leaf shape, with two island populations exhibiting more deeply lobed leaves than those from the mainland. Andersson [72] used a crossing experiment to demonstrate that while deep lobes on one island are caused by a single dominant locus, on the other island they are caused by multiple loci, which suggest an independent origin of the trait on each island. Further work is needed to confirm this independence, to elaborate the adaptive value of leaf dissection in this system and to establish if there is any reproductive isolation other than geographic between the deeply lobed and less lobed forms.

A.10. *Deschampsia cespitosa*. In the 1970s, this perennial grass colonized metal-contaminated soil at two locations in Southern Ontario, Canada. Isozyme analysis of the populations at both contaminated sites, as well as uncontaminated sites to the south, found reduced variability in the metal-contaminated populations [73]. Unique alleles in each contaminated site suggested that each had an independent origin. However, a more recent genetic marker analysis with the same populations has produced equivocal results, indicating that although there are two origins for the contaminated site populations, one population at one site shares its origin with all populations at the other contaminated site [74]. No work has been done on barriers to gene flow between populations or on the mechanisms of heavy metal adaptation.

A.11. *Eucalyptus globulus*. Three populations of *Eucalyptus globulus* that inhabit exposed granite headlands in southeastern Australia have a dwarfed morphology and flower earlier than their tall ancestors [75]. Relatedness analyses using several nuclear and chloroplast markers show that the dwarfed populations are more closely related to the nearest population of the tall ecotype than to each other [75]. Observations of progeny allele frequency show no evidence of pollen-mediated gene flow from the much more abundant tall ecotypes to the dwarf ecotypes [75]. This suggests that there have been at least three independent transitions to
dwarfism in the novel exposed granite headland habitat (barring, of course, a long history of introgression after a single origin and dispersal). This case is quite promising, as it has strong evidence for both independence and isolation from ancestral populations. What remains is to demonstrate the compatibility of the dwarf populations with each other, and to more clearly elucidate the adaptive value of dwarfism in this system.

A.12. Geonoma macrostachys. Lowland forests in Peru are home to two subspecies of the palm Geonoma macrostachys that are alternately more abundant in flood plain versus tierra firme habitat [76]. The two subspecies differ in leaf shape and are reproducibly isolated by phenology, flowering activity, and pollinator spectrum [77]. However, ISSR variation strongly partitions among sympatric populations of both subspecies rather than between the subspecies, and subspecific genetic classification is not possible [78]. In three different forests, Roncal [78] found consistently strong microhabitat preferences for each of the two subspecies, which, along with the genetic data, suggest an independent origin of the subspecies in each environment. Alternate hypotheses of a history of local gene flow among subspecies or phenotypic plasticity must be ruled out before this case can be considered parallel speciation, and further work on reproductive isolation and the mechanisms of microhabitat adaptation is warranted.

A.13. Hemerocallis citrina var. vespertina. On Japanese archipelagos, there appear to be three independent origins of nocturnal flowering and associated changes in floral morphology in Hemerocallis citrina var. vespertina from the whole-day flowering H. flava. Data from three chloroplast markers place H. citrina var. vespertina within three different geographically distinct subspecies of H. flava from mainland Asia, despite persistent morphological and phenological differences between the two species [79]. This could be the result of introgression leading to chloroplast capture, or incomplete lineage sorting of ancestral variation, and little is known about reproductive barrier strengths within the three clades of H. citrina var. vespertina or between the two species. Further study is needed to differentiate these hypotheses and elucidate reproductive isolation in the system, as well as the adaptive mechanism underlying variation in floral phenology.

A.14. Hieracium umbellatum. Possibly the first observed case of parallel ecotypic differentiation, Swedish Hieracium umbellatum, was described by Turesson in 1922 [80]. His study found that dun inhabiting plants produced more prostrate stems and thicker leaves than those in open woodlands and that these differences were heritable. Furthermore, although these ecotypes shared many morphological traits, they also retained some leaf characters more like those of neighbouring populations of a different ecotype than distant populations of the same ecotype. Ecotypes also differed in flowering time, an early-acting reproductive barrier. This case is promising, but modern population genetics should be used to confirm the phylogenetic independence of these populations, and further work needs to characterize the adaptive mechanisms underlying the ecotypic characters and the extent of reproductive isolation between and within ecotypes.

A.15. Lasthenia californica. The common goldfield, Lasthenia californica, grows in a variety of habitats and has two flavonoid pigment races that strongly correlate with edaphic tolerance. Race A grows on ionically extreme habitats such as coastal bluffs, alkaline flats, vernal pools, and serpentine soil, while race C is found on ionically benign and drier locations such as pastures and oak woodlands. Phylogenetic analyses using ribosomal and chloroplast sequences along with allozyme variation indicate two cryptic clades within the species with representatives of both races in each [81–84], suggesting a parallel origin of each race. Greenhouse experiments indicate that race A plants, regardless of phylogenetic clade, have greater tolerance to Na⁺ and Mg²⁺ and in drought conditions race C plants flower earlier and produce more flower heads [85, 86]. Preliminary data shows reduced seed set between different races of the same clade and greater pollination success between populations of the same race during interclade crossing, although these data have not been formally published after being presented in Rajakaruna and Whitton [87]. This case has great potential, but further conclusions await stronger published evidence.

A.16. Microseris lanceolata. Australia is home to two ecotypes of Microseris lanceolata: a “murnong” ecotype found below 750 m elevation which produces tubers, and an “alpine” ecotype found above 1000 m elevation which reproduces vegetatively in addition to having a significantly later flowering time [88]. Phylogenetic analyses based on chloroplast markers show three geographically correlated clades within M. lanceolata that all include individuals of both ecotypes, suggesting parallel independent origins [89]. Nuclear AFLP markers also support this hypothesis, as genetic distance among populations correlates strongly with geographic distance rather than ecotype identity [90]. This pattern may be explained by a single origin and dispersal of each ecotype followed by significant local hybridization between ecotypes, but Vijverberg et al. [90] emphasize that these populations have managed to maintain their ecotypic characteristics even in the face of gene flow. Given this and evidence that crosses between and within ecotypes are viable, it seems likely that selection is acting in parallel to maintain or recreate fixed differences between these populations.

A.17. Petunia axillaris. Petunia axillaris has likely repeatedly evolved white flowers from ancestral colored flowers, as indicated by sequence data showing 6 different loss-of-function mutations of the ANTHOCYANIN2 (AN2) gene in wild P. axillaris populations [91, 92]. It is possible that AN2 was downregulated a single time and that the loss of function mutations occurred subsequently, but P. axillaris does not exhibit the low expression of AN2 that would be expected if the AN2 promoter was inactivated [92].
Furthermore, pollination experiments using introgression lines and transgenic flowers have shown that functional and nonfunctional AN2 alleles have a large effect on pollinator visitation, which is likely a strong reproductive barrier in this system [92]. However, we do not yet have evidence that these floral colour transitions have been driven by natural selection, and there is only weak evidence for directional selection at these loci [92]. It is certainly possible that this is a case of repeated adaptation to a new pollination syndrome, but this remains to be tested.

A.18. Plantago maritima. This widespread plant grows on inland, coastal, and salt marsh habitats across North America and Europe. In eastern North America, salt marsh plants have relatively lax spikes when compared to plants in rocky habitats. Similarly, British coastal plants also have lax spikes relative to inland populations, although in this case the spikes are less dense than North American salt marsh plants [93]. Although these traits appear to have evolved independently and in parallel on two different continents, little is known about the genetic basis of these traits or their effects on reproductive isolation or local adaptation.

A.19. Poa annua. Annual bluegrass (Poa annua) is an agricultural weed with populations known to be resistant to the triazine herbicides [94]. Isozyme work has found equivalent levels of variability between resistant and nonresistant populations, suggesting ongoing gene flow after the founding of resistance [95]. Although herbicide resistance is a relatively simple trait to evolve (often requiring a single amino acid change), there is no evidence to suggest independent evolution of resistance in this species beyond the geographic distance between resistant populations and no evidence that triazine resistance is involved in reproductive isolation.

A.20. Schizanthus grahamii. Two closely related Andean butterfly flowers are taxonomically differentiated by pollination syndrome, floral morphology, and mating system: Schizanthus hookeri is purple flowered, bee pollinated, and highly outcrossing, as are other species in the genus, while S. grahamii is capable of self-fertilization, primarily hummingbird pollinated, and exhibits several color morphs. The two taxa are rarely found growing sympatrically despite overlapping elevational ranges (with S. grahamii generally at higher elevations). Within one sympatric population, experimental interspecific crosses produced no seed set, while intraspecific seed set was 63–72% [96]. Chloroplast sequence data support two independent parallel origins of the S. grahamii morphotype: a southern clade characterized by red flowers that shares haplotypes with southern populations of S. hookeri, and a northern clade with yellow or pink flowers that shares haplotypes with the northernmost S. hookeri populations [96]. However, this pattern could be explained by historical hybridization followed by chloroplast capture, and further work needs to be done to rule out this possibility and characterize gene flow and reproductive barriers between the two S. grahamii clades.

A.21. Silene dioica. The red campion, Silene dioica, has the ability to colonize both serpentine and nonserpentine habitats. Although Westerbergh and Saura [97] demonstrated using isozymes that serpentine populations tended to group with neighbouring nonserpentine populations, indicating multiple origins of serpentine tolerance or possibly ongoing gene flow, later work showed that all populations, regardless of soil type, had serpentine soil tolerance [98]. Thus, serpentine tolerance in Swedish S. dioica is likely constitutive and not parallel.

A.22. Silene vulgaris. At mine sites across Europe, Silene vulgaris from two subspecies (ssp. maritima in coastal and ssp. vulgaris in continental Europe) has acquired tolerance to high levels of zinc and copper. Complementation tests between sites indicate that zinc tolerance is governed by two loci, both acting in highly tolerant populations of both subspecies [99]. In one mildly tolerant population, zinc tolerance appears to be controlled by only one of the tolerance alleles, and intolerant populations in both subspecies have neither. Similarly, copper tolerance is controlled at two loci: one common across all tolerant populations and a second found only in Innsbruck, Germany where plants are extremely tolerant [100]. The presence of populations with a variable genetic basis for tolerance in two subspecies at multiple sites across Europe may represent parallel adaptation, but the phylogenetic independence of these populations has not been confirmed, and no studies of reproductive isolation in the system have been completed. At minimum, populations of both subspecies lack strong postzygotic barriers, as complementation tests are possible. Further work to understand the population genetics of metal tolerance (from ancestral variation, repeated novel mutations, or gene flow between metalliferous sites) should also be done. Additional populations of S. vulgaris have colonized naturally metalliferous (serpentine) soils in Switzerland and differently contaminated mine sites in Canada and Europe [101, 102], which may indicate the ease of evolving metal tolerance in this species.

A.23. Streptanthus glandulosus. The Streptanthus glandulosus complex contains several subspecies endemic to serpentine outcrops in California. Although a majority of populations are found on serpentine soil, nonserpentine populations are also present. Kruckeberg [103] tested serpentine and nonserpentine populations of S. glandulosus on serpentine soil and found that nonserpentine populations were serpentine intolerant, although this study only qualitatively examined growth rate due to technical problems. Later studies used cpDNA restriction site data and ITS sequence to show that the species is structured into several roughly geographically based subspecies [104–106]. Nonserpentine populations occur in multiple subspecies and are more closely related to nearby serpentine populations rather than further nonserpentine populations. This suggests that serpentine intolerance, as well as perhaps greater competitive ability on nonserpentine soil, has occurred multiple times in this species complex. Crossing experiments in this complex
found that hybrid fertility is inversely related to geographic distance, suggesting that nonserpentine populations would be more compatible with neighbouring serpentine populations than distant nonserpentine ones [107]. More study is needed to determine if the change in edaphic tolerance is associated with a change in compatibility, a condition necessary for parallel ecological speciation. Additionally, the serpentine intolerance of nonserpentine populations should be reevaluated in a more quantitative manner.

B. Descriptions of Frequently Cited Examples of Parallel Speciation in Animals

B.1. Astyanax cave fishes. In northeastern Mexico, fish of the Astyanax species complex have repeatedly adapted to cave environments. A recent study incorporating mitochondrial and nuclear markers supports at least two independent origins of cave-adapted Astyanax [27]. One genetic cluster is associated with older cave populations characterized by highly reduced eyes and pigmentation, while another is shared by many surface populations and putatively more recent cave-adapted populations with less extreme phenotypes. Nevertheless, all cave populations will interbreed in the laboratory and share many adaptations to a subterranean environment, including an increase in taste bud number, improved lateral line sense, and greater fat storage ability as well as reduced pigmentation and eyes. Although surface and cave fish will also cross in the laboratory, there is no genetic evidence of recent hybridization between the two groups in most populations. In one location, surface fish are even regularly swept into a cave by flooding—yet this cave population shows very little genetic admixture, and only two intermediate forms have ever been found despite repeated sampling [27]. In another cave with frequent introductions of surface fish, fish without a cave-adapted phenotype have been observed starving to death and being eaten by fish with cave-adapted phenotypes [27]. Yet in lighted conditions in the laboratory, surface fish outcompete cave fish for food. Taken together, the evidence is quite strong for at least two independent parallel adaptations to caves by Astyanax, and although reproductive isolation in the system may be primarily extrinsic it is reciprocal and appears quite effective.

B.2. Coregonus spp. Whitefish is potentially undergoing several parallel speciation events. The North American lake whitefish, C. clupeaformis, is present in at least six lakes in both a “dwarf” limnetic form and a larger-bodied, “normal” benthic form [108]. Geographical isolation during the last glaciation is reflected by three ancient mitochondrial lineages, likely without much morphological divergence [13, 109]. The data suggest that subsequent secondary contact (<15,000 years) of these lineages gave rise to parallel, independent sympatric populations of the two ecotypes [108], at least some of which exhibit strong intrinsic and extrinsic postzygotic reproductive isolation [110]. In addition to body size, these ecotypes differ in gill-raker number, age at maturation, relative fecundity, growth rate, and swimming behavior [108]. A number of quantitative trait loci for growth rate and morphology have been identified as under divergent selection between the ecotypes [111, 112]. Further research has demonstrated parallel changes in gene expression among independent sympatric ecotype pairs [113], while changes at the genetic level between normal and dwarf populations are more weakly correlated among lakes [112, 114]. Changes in expression in at least two candidate genes are also replicated in the closely related European whitefish system ([115], see below).

In a series of Northern European lakes, European whitefish (C. lavaretus) has differentiated into two ecotypes: a “sparsely rakered”, larger-bodied, benthic form, and a “densely rakered” smaller limnetic form. Populations of this species form three ancient mitochondrial clades, which do not correlate with and are more ancient than gill raker divergence [116]. The divergent traits in these populations are highly bimodally distributed, and are strongly correlated with habitat use and diet [14, 117]. These morphological relationships contrast with genetic relationships—where ecotypes within a single lake cluster more closely with each other than with fish of similar morphology in other lakes [14]. These lakes are less than 15,000 years old. Although phenotypic and genetic differences in both the North American and European species complex are well characterized, evolving in parallel, and show signatures of divergent selection, comparatively little is known about the strength and nature of reproductive barriers in both systems.

B.3. Gasterosteus aculeatus. Threespine sticklebacks seem particularly prone to parallel evolution, with several well-documented parallel transitions between environments and foraging niches. Throughout the Northern Hemisphere (and in Japan), large-bodied anadromous threespine stickleback have repeatedly evolved into smaller stream-resident fish [17, 131]. These freshwater transitions involve the parallel fixation via natural selection of low-armor, reduced pigmentation, and pelvic loss alleles in multiple independent populations [131–133]. In the case of pelvic loss, these populations exhibit at least three different mutations at the same locus—incontrovertible evidence for the independent origin of this adaptation [133]. In addition, microsatellite data reveals that the majority of genetic variation is partitioned regionally rather than between ecotypes [17]. In the lab, stream-resident fish are more than twice as likely to mate with other stream-resident fish than with anadromous fish (and vice versa), even when from populations as distant as Iceland and Japan [17].

Stickleback have also transitioned in parallel between lake habitats and local streams at least 6 times independently in British Columbia and likely elsewhere, as demonstrated by a number of genetic analyses [123–125]. This transition involves a substantial shift in prey availability and abiotic environment, and the ecotypes differ in body size, shape, and foraging behavior [124–126]. In one British Columbian lake-stream system, common garden experiments confirmed the genetic basis of these traits, a reciprocal transplant experiment showed a weak reduction in growth rate for lake
and inlet fish enclosed in the other habitat, and release-recapture studies demonstrated a bias towards recapture of inlet fish in the inlet [126]. Taken together, this is relatively weak evidence for isolation and selection, but other lake-stream systems may have evolved stronger barriers between ecotype pairs [124].

Possibly the best-studied case of potential parallel ecological speciation is the independent origin of “benthic” and “limnetic” ecotypes in at least five British Columbian lakes [15, 24]. These ecotypes specialize in foraging niche, with a number of morphological and behavioral differences, and exhibit both prezygotic behavioral and extrinsic postzygotic barriers to gene flow [15, 16, 118]. Genetic and biogeographic data support the independent colonization of each lake [15, 119, 120]. Each ecotype is more likely to spawn with fish of the same ecotype than of the other ecotype, regardless of lake of origin [15]. Limnetic backcross hybrid fish grew twice as fast as benthic backcross fish in a limnetic environment, and vice versa for benthic backcrosses in a benthic environment [118].

In all of these threespine stickleback transitions, reproductive isolation appears to have evolved via assortative mating by body size acting concurrently with divergent selection on body size [3, 16, 23]. However, parallel cases vary widely in the strength of reproductive isolation and genetic differentiation between local ecotypes (e.g. [125, 127]). In some cases, evidence for reproductive isolation is conflicting despite evidence for genetic differentiation [128–130]. Researchers have been able to manipulate mating preferences in stickleback by rearing juveniles with individuals of the other ecotype, indicating that reproductive isolation is at least partly extrinsic [121]. Additionally, at least one independent case of “benthic” and “limnetic” ecotypes has collapsed back into a single panmictic pool, possibly due to the human-mediated introduction of an exotic crayfish [122].

B.4. Littorina saxatilis. The marine snail Littorina saxatilis has repeatedly evolved pairs of ecotypes on the rocky coasts of Northwestern Europe [19]. Although we only discuss one here, there are several regional cases of this divergence, with ecotypes adapted to different microhabitats created by tidal and substrate variation. The best-studied ecotype pair has evolved at least three times independently in Galician Spain, where the two types specialize in and prefer either the high intertidal barnacle belt or the low intertidal mussel belt and occasionally hybridize in the intermediate area between those environments [134, 135]. The main axes of morphological differentiation are in shell size and shape, which aid in resistance either to dislodging by wave action (thin, small shells with large apertures for the muscular foot) or to crab predation and desiccation stress (thick, large shells with small apertures), and there are additional differences in shell ornamentation [19]. These characters are heritable, and genetic variation partitions primarily among independent beaches rather than between ecotypes, although the ecotypes do appear genetically divergent on a local scale [136, 137]. Mitochondrial data similarly support multiple independent origins of the ecotypes [138]. In contrast, several candidate loci discovered in an FST outlier screen appear to be under divergent selection between ecotypes in multiple populations [139]. In a reciprocal transplant experiment, each ecotype survived at much higher rates in its native microhabitat than either the other ecotype or hybrids between them [134]. A later reciprocal transplant demonstrated a strong correlation between the divergent morphological characters and survival in each environment, further evidence for the role of selection in maintaining ecotypic differences [140]. These ecotypes exhibit assortative mating by body size that, along with immigrant inviability and habitat preferences, acts to reduce but not eliminate gene flow between ecotypes [18, 141].

B.5. Timema cristinae. These western North American walking-stick insects specialize on two host plant species and exhibit two different color morphs that are reciprocally more cryptic on different host plants [142]. The two color morphs are found in higher proportions on the host plant species on which they are most cryptic, although this is quite variable for individual plants, and cryptosis helps the insects to avoid strong predation pressure by birds and lizards [142, 143]. The color morphs also differ in average body size, host preference, and cryptic resting behavior, although again this is quite variable for individual populations [20, 143]. In one study, each color morph copulated more readily with the same color morph than the other morph, regardless of the population of origin [20]; however, there is no clear relationship between morph-specific divergent characters and reproductive isolation [143]. Phylogenetic analyses show that the color morphs are not monophyletic [20, 144], indicating possible multiple origins of at least one of the morphs, although this pattern could also be explained by a single diversification event followed by ongoing local gene flow between the morphs. One of these studies also demonstrated using an outlier approach that a small number of loci are under divergent selection between the color morphs [144]. Consequently, divergent selection appears to be acting in parallel in many independent populations. Indeed, as discussed by researchers in this system, these walking-stick insects seem to be experiencing a heterogeneous balance of gene flow and divergent selection, and it is unclear whether this process will ultimately result in speciation between the currently weakly isolated morphs.

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References


Review Article

From Local Adaptation to Speciation: Specialization and Reinforcement

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Local adaptation is the first step in the process of ecological speciation. It is, however, an unstable and dynamic situation. It can be strengthened by the occurrence of alleles more specialized to the different habitats or vanish if generalist alleles arise by mutations and increase in frequency. This process can have complicated dynamics as specialist alleles may be much more common and may maintain local adaptation for a long time. Thus, even in the absence of an absolute fitness tradeoff between habitats, local adaptation may persist a long time before vanishing. Furthermore, several feedback loops can help to maintain it (the reinforcement, demographic, and recombination loops). This reinforcement can occur by modifying one of the three fundamental steps in a sexual life cycle (dispersal, syngamy, meiosis), which promotes genetic clustering by causing specific genetic associations. Distinguishing these mechanisms complements the one- versus two-allele classification. Overall, the relative rates of the two processes (specialization and reinforcement) dictate whether ecological speciation will occur.

1. Introduction

The debate over speciation is not new [1–7] and is a complex subject as speciation represents a “cluster of theories woven from many strands” [7] that can be approached from different angles: sympatry versus allopatry, intrinsic versus extrinsic selection, premating versus postmating isolation, isolation versus adaptation, one-allele versus two-allele mechanisms, primary versus secondary contact, genic versus genomic, and so forth. This diversity of possible approaches can even lead to a synthesis based on the idea of a menu with different possible options for each course [8].

One view has reemphasized Darwin’s view that speciation was about adaptation to different habitats or niches [2, 4, 9, 10]. At a microevolutionary scale, this process often starts within species as local adaptation. This can be the beginning of future divergence and eventually speciation. However, the basic process of local adaptation is often seen as too “preliminary” to stand at the core of a theory for speciation. Indeed, “mere differential adaptation (…) does not constitute species” [1] and hybrid unfitness is often the main selective scenario envisioned to be necessary. Hybrid unfitness can be caused by alternative adaptive peaks in the same habitat, whereas local adaptation usually occurs with a single peak that differs in different habitats, which is quite different, even if not always recognized as such [1, 8, 11]. Of course, both phenomenon are not exclusive and evaluating their relative importance is often controversial [12], as both produce indistinguishable spatial patterns [13]. We will see that the route from local adaptation to speciation has been explored in a somewhat separate theoretical corpus that still has to be fully incorporated to a microevolutionary view of speciation.

Environmental heterogeneity is pervasive and local adaptation is the direct selective consequence of different selection pressures occurring in different places [14]. Local adaptation—the greater average fitness of local individuals compared to immigrants—occurs whenever the “grain” of habitat is sufficiently coarse compared to the scale of dispersal [15, 16]. It has various consequences ranging from niche evolution, evolution of specialization, to ecological speciation. In the context of speciation, local adaptation is a selective context that is universal: it applies to sexual or asexual species alike, contrary to selection pressures caused
by hybrid incompatibilities. However, it also has specific features. First it is a dynamic and gradual process. Local adaptation can constantly strengthen if new alleles better adapted to local conditions arise and increase in frequency, or disappear if generalist genotypes spread or if differentiation is swamped by gene flow. In contrast, a situation of secondary contact is not a gradual process, but often the abrupt exposure of genetic incompatibilities that have accumulated independently in allopatry. It results in tension zones that can be stable over long periods of time [17]. Second, hybrids between two locally adapted parental genotypes are often not the worst genotype in any of the environments. With local adaptation, there is not selection against hybrids per se: there is selection against alleles or genotypes that are not locally favorable. This distinction has important consequences understanding how reinforcement works in the context of local adaptation compared to the case of reinforcement to avoid producing unfit hybrids. Here, I use reinforcement sensu lato to mean the evolution of premating isolation resulting from selection against hybrids or locally maladapted genotypes [18]. In this paper, I will focus on these two topics and try to show that their outcomes are often less straightforward than early models have suggested, and that the theory of local adaptation has to be more fully integrated into a global view of speciation and diversification.

2. Topic 1—The Dynamics of Local Adaptation

Because niche specialization can occur in the presence of gene flow in primary contact and does not require time in isolation to evolve genetic incompatibilities, it results in a more dynamical process, where further niche specialization may or may not occur, with direct consequences on reinforcement towards more isolation.

2.1. Habitats. Models of local adaptation and ecological speciation most often start by assuming that there are different habitats exchanging migrants (with the rate of migration corresponding to the cases of sympathy, parapatry and allopatry [3]). However, the definition of habitat can be problematic. Field ecologists would certainly define it by a combination of biotic/abiotic conditions in a landscape. For instance, when thinking about land plants, they would categorize soil type, moisture, temperature, light, slope, disturbance regime, and so forth. From an ecological genetics perspective, a first difficulty is to define these variations at the relevant scale, that is, at the scale of dispersal of the focal species [15, 16]. A second difficulty is to account for distance. Because dispersal is most often distance limited, the spatial configuration makes a difference in terms of habitat definition. This is well known in complex or mosaic habitats [19], but is true even in very simple landscapes [20]. A given ecological condition will be less prone to local adaptation if close to a habitat boundary than if surrounded by identical conditions. Boundaries may even favor the local evolution of generalists, something that contradicts the simple competition exclusion principle [20]. Finally, conditions also vary through time, which strongly limit the scope for the long term maintenance of locally specialized genotypes. Overall, locally adapted genotypes are unlikely to arise “on every bush” [21], yet are expected to arise frequently.

2.2. Local Adaptation and the Origin of Tradeoffs. One basic process by which local adaptation arises is when, within a large enough habitat, an allele increases in frequency that is beneficial inside but deleterious/neutral outside this habitat. The condition for this increase in frequency is determined by the strength of selection inside and outside, the size of the pocket relative to the scale of dispersal and by possible gene flow asymmetries between habitats [15, 22]. Any allele with a sufficient benefit inside will increase in frequency no matter if it presents strong deleterious effects outside. This process does not necessarily favor alleles exhibiting little antagonistic effect across habitats; anything goes that is sufficiently favorable locally. At the same locus, allele replacement can occur in both directions, favoring either stronger or weaker specialization [23]. Another possibility is that neutral mutations drift at high frequency locally despite being deleterious elsewhere [14, 24, 25]. However, even if this can occur [26], it requires very limited gene flow and may be globally less conducive to strong local adaptation.

This process has no reason to stop and lead to a process of “amelioration” [27] whereby new favorable alleles replace previous ones at a given locus [23, 28–30], modifier alleles at new loci evolve to correct for deleterious side effects of previous ones [31–33], duplications and new functions can arise [34–36], and so forth. The question is whether this amelioration will lead to the evolution of generalist genotypes that can accommodate all habitats or whether local adaptation will strengthen and lead to specialized ecotypes that have diverged at a large number of loci. The answer is not straightforward. The first approach is to build a model imposing a trade-off curve between habitats. Whether specialists or generalists evolve depends then on the shape of this trade-off curve [37, 38]. Globally speaking, more concave curves facilitate the evolution of specialists, whereas more convex ones favor generalists, and sometimes both can coexist [37, 38]. However, there is no clear ultimate reason for choosing one curve over another or not allowing these trade-off curves to evolve as well. Another approach that has been much less explored would be to introduce a distribution of mutation effects, where specialist mutations are much more common than generalist mutations (having to solve the problem of a single habitat) and are constantly appearing at different loci maintaining local adaptation. In the latter situation, and if no other constraint is involved, the outcome in the very long run would be nearly perfect adaptation to the different habitats. There are however three positive feedback loops that are likely to interfere with this outcome and favor increased specialization.

2.3. The Demographic Feedback Loop. The first feedback is demographic. As far as local adaptation causes a local increase in density, it will also make life easier for more specialist alleles to increase in frequency. This is due to
the fact that density differences cause asymmetric gene flow, which gives an advantage to alleles favored in the denser habitat [14, 22]. Because of gene flow, an allele too detrimental outside the habitat where it is favored may be unable to increase in frequency. Despite having the potential to contribute to specialization if the habitat was isolated, it remains at mutation-selection balance. Such alleles may be very common; I term them “contending” alleles. If density becomes higher in this habitat, contending alleles may now be able to increase in frequency and contribute to local adaptation. This increase in density is likely to occur at least in some cases when local adaptation takes place. The positive feedback loop occurs because a local increase in density causes more and more alleles that are locally beneficial to be recruited, which strengthens local adaptation, increases local density and facilitates further the increase in frequency of other locally beneficial alleles (the reverse can also occur, which is known as migration meltdown [14, 39]). Because the ratio of density is as effective as the square of selection ratio inside versus outside the habitat [14, 22], this effect is likely to be strong in natural populations. Conversely adapting to a sink habitat makes it very difficult for the same reason as shown by niche expansion models [40–42].

2.4. The Recombination Feedback Loop. The second feedback loop is due to indirect selection among loci directly involved in local adaptation. When a locally beneficial allele increases in frequency, it will favor the spread at closely linked loci of other locally beneficial alleles. This is due to the fact that dispersal generates linkage disequilibrium between loci that share a similar frequency variation across habitats, as expected for two loci involved in local adaptation to the same habitat. This linkage disequilibrium translates into indirect selection that mutually benefits the locally adapted alleles at the two loci [43, 44]. For instance, a contending allele could start to increase in frequency if it becomes sufficiently linked to another locus involved in the local adaptation. This phenomenon generates a positive feedback loop within the genome where locally adapted alleles are more likely to be recruited in genomic regions already harboring a previous locus involved in the local adaptation. It can generate “genomic islands” of local adaptation that extend further and further [45, 46], a specific process that can gradually produce strong genetic divergence at many contiguous loci in linkage disequilibrium [1, 47].

2.5. The Reinforcement Feedback Loop. The third feedback loop is due to reinforcement, that is, the evolution of traits promoting premating isolation between differentially locally adapted genotypes. Reinforcement tends to make life easier for locally beneficial alleles: it allows more alleles contributing to local adaptation to be recruited and locally beneficial alleles to reach higher frequencies. For instance, contending alleles could be recruited if habitat choice started to evolve. In effect, habitat choice minimizes the possible negative fitness that an allele can have in habitats that are different from the habitat where it is favored. Thus, reinforcement is likely to promote increased specialization. Reciprocally, strong local adaptation increases the selection pressure to reinforce it, so that both phenomena can act in concert in a positive feedback loop [9, 37, 38, 48].

2.6. The Relative Dynamics of Local Adaptation and Reinforcement. Ultimately, ecological speciation will result only if reinforcement occurs quickly enough compared to the evolution of generalists and the breakdown of local adaptation. The different feedback loops mentioned above will tend to favor this outcome, but may not be strong enough to lead to speciation. For instance, the evolution of habitat choice, reduced dispersal, selfing, and so forth, can strongly reduce the chance that a generalist allele would spread, but the actual outcome depends on the relative rates of reinforcement and loss of specialization. This dynamical issue is not something that has been fully appreciated in the context of ecological speciation (but see [38] in the context of a fixed tradeoff). It differs from the situation of reinforcement in a tension zone by the fact that it is less stable and very sensitive to several feedbacks. More work is certainly needed to clearly delineate the conditions favoring speciation in this context.

3. Topic 2—The Reinforcement of Local Adaptation

“Individuals that have parents selected in the same habitat and that stay in that habitat are more likely to have genes appropriate to that environment than another randomly chosen individual. Thus, mating locally and staying in the same habitat is always favored from the point of view of a continually evolving genome” [25].

In the early 70s, several models have addressed the problem of reinforcement of local adaptation. The first was proposed by Antonovics [49]. This model showed that, because mating at random is risky in the context of local adaptation, evolution favors that like mates with like and that selfing is even safer to maintain local adaptation. Then Balkau and Feldman [50] showed that, in the context of local adaptation, migrating, or sending offspring elsewhere is likely to decrease an individual’s fitness or that of its offspring. With a modifier model, they showed that this effect caused indirect selection to reduce dispersal as much as possible. Finally, Slatkin [43] suggested and D. Charlesworth and B. Charlesworth confirmed [51] that sex and recombination is likely to break combination of genes that have been locally selected for and should be selected against in the context of local adaptation. These findings have since been constantly reported or given as examples of “one-allele” mechanisms that are likely to drive the evolution of isolation in parapatry. (The one-allele versus two-allele classification refers to cases where a single or two different alleles spread at the modifier locus to promote genetic clustering [21]. This classification is very useful despite leading to some complications (see [52] and Table 1 note 7)). In this section, I will reconsider these conclusions in the light of more recent models on the evolution of assortative
mating [53], dispersal [54, 55], and recombination [11, 45, 56] in the context of local adaptation. Contrary to what is commonly thought [8, 21, 57], I will show that these one-allele mechanisms do not inevitably lead to speciation, even in the absence of direct cost. I will then make a comparison of the underlying mechanisms and propose a typology of cases (orthogonal to the one- versus two-allele classification) that may prove useful understanding and modeling speciation (Table 1).

Before proceeding, we note that several important findings have also been made regarding this process since these early models. First, the role of ecological-based adaptation in speciation has received considerable support in the last decade [2, 4, 9, 10]. Second, several empirical findings have supported the idea that reinforcement could indeed occur in the context of adaptation to different habitats [49, 58–67] or at least that there is often ample opportunity for reinforcement [6].

3.1. The Evolution of Selfing and Assortative Mating. The evolution of nonrandom mating has been extensively studied in the context of reinforcement and reproductive isolation [18, 68–70]. However, it has also been extensively studied to understand the evolution of mating systems within species [71–73]. Interestingly, the two approaches are usually considered separately and emphasize completely opposite outcomes. The first predicts the evolution of more assortative mating with increased outbreeding depression or hybrid unfitness. The second predicts the evolution of less assortative mating or selfing with increased inbreeding depression. The models studying reinforcement include outbreeding but not inbreeding depression [18, 70, 74–77] while the models studying mating system evolution do exactly the opposite [71–73].

Local adaptation causes outbreeding depression if different alleles are favored in different habitats [78], which is the reason why it is widely thought that spatially heterogenous

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<th>Table 1: Classification of reinforcement mechanisms based on the life stage modified and their consequences on genetic associations involved in genetic clustering. This classification is orthogonal to the one- versus two-allele classification.</th>
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¹This would also apply to loci involved in genetic incompatibilities in a secondary contact.
²Unless mating is selective and causes a direct advantage to locally adapted alleles (i.e., it changes frequency at the local adaptation loci), as found in model involving sexual selection [8, 103].
³Notation as in [104, 105], where m is the modifier locus and a, b the local adaptation loci. “Primary” association refers to the fact that the phenotypic effect of the modifier causes first a direct change on the genetic composition of the population (on frequency, within or between loci associations), which may then change the efficacy of selection. Eventually, a modifier promoting clustering will end up associated to the beneficial allele locally (Cma,Ø > 0). See Figures 1, 2 and 4 for examples.
⁴Indirectly by increasing the variance in fitness and the efficacy of selection.
⁵Besides possible direct costs relative to the strategy used (e.g., cost of finding a mate or the right habitat). Different traits are exposed to a variety of other selective effects (see text).
⁶Which generates inbreeding depression.

7 Phenotype 1 and 2 may result from alleles at the adaptation locus or to another unrelated marker trait. Similarly in a “one-allele” model, self-similarity may be evaluated in reference to a marker trait at another locus than the modifier or the local adaptation locus. In both cases, the marker trait has to diverge in the two incipient species, which is essentially a two-allele mechanism. Thus, with three locus like this, the one- versus two-allele distinction is made more complicated by the fact that the marker trait must diverge (two-allele), but the modifier of the strength of assortment need not (it can be one- or two-allele) [52]. Another complication of the one- and two-allele classification arises when the locus exposed to postzygotic selection also causes premating isolation (as seen in so-called “magic trait” models). This can be thought as the limit where the loci causing prezygotic isolation and postzygotic selection become confounded.
Figure 1: Indirect selection on a selfing/assortative mating modifier with local adaptation [53]. Sketches how a selfing or assortative mating modifier evolves in presence of local adaptation (for the sake of illustration, the S allele causes maximal assortment and s specifies random mating). Here assortment can be produced by selling or assortative mating based on the genotype at the local adaptation locus (A mates with A and a with a). Before migration (step 1), consider two habitats with haploid individuals. On the left A allele is favored at a local adaptation locus, whereas a is favored on the right. To make things simple, we consider these alleles to be fixed where they are beneficial. At step 2, migration occurs between habitats (with $m = 1/2$, migrants in red). Then syngamy occurs in each habitat. The small circles represent diploid individuals. In each habitat, one can distinguish the subpopulation with full S-assortment allele (4 individuals on the left) and random mating s allele (4 individuals on the right). Importantly, at this step the S allele becomes positively associated to extreme aa and AA homozygotes (thus, variance in fitness is greater in the subpopulation with the S allele). Finally, selection occurs (favoring A on the left and a on the right, very strongly but in a codominant way in the illustration). At the end of this generation, the modifier has not changed in frequency (it is still 1/2). Yet, selection has generated LD between the random mating s allele and the locally inferior allele locally (the inferior allele is only found on the same chromosome as s in each habitat, orange dot). At the next generation, this LD will persist (it is decreased at most by one half by a round of free recombination) and cause indirect selection in favor of S. Note that if the locally beneficial allele is recessive (all heterozygotes eliminated on the right and the left), we see that direct selection occurs favoring S (its frequency rises to 2/3 on the sketch), but that less LD is generated. Exactly the opposite occurs if the local beneficial allele is dominant.

Selection favors the evolution of assortative mating by a one-allele mechanism in the absence of direct costs [8, 18, 21, 68, 69, 79, 80]. However, the dominance relationship of locally adapted alleles within each habitat may also cause inbreeding depression which can, in fact, prevent the evolution of premating isolation. When both phenomena act in concert, the outcomes vary tremendously depending on parameter values [53], and more assortment is not necessarily favored even in presence of strong local adaptation. In fact, because a polymorphism at a locus involved in local adaptation is more easily maintained when locally beneficial alleles are dominant, less assortment may often be favored in natural situations with local adaptation [53]. There are theoretical reasons for expecting such dominance relationships between locally adapted alleles [81]. Because theoretical models of reinforcement have rarely considered the case of local adaptation, and when they did, considered only haploid or diploid with particular dominance [18, 70, 82], these conclusions have remained largely overlooked. There are numerous mechanisms of assortment [69] and each can evolve slightly differently. For instance when local adaptation is based on a conspicuous trait (shell thickness in Littorina [65], coloration in Chrysopa [62] or Heliconius [83], etc.), mate choice can be cued directly on this trait, which is very efficient unless the right mate is rare and difficult to find in the population [84]. Another simple way to mate with a self-similar phenotype is to self-fertilize when hermaphrodite which is also very efficient, does not incur the cost of finding the right mate and does not require the ability to discriminate the locally adapted trait. In both cases, local adaptation
3.2. The Evolution of Dispersal. Individuals that have survived until reproduction have genotypes that work relatively well where they are. Because of environmental heterogeneity, migrating or sending offspring elsewhere is likely to decrease fitness. Local adaptation indeed generates an indirect selection pressure in favor of less dispersal \([50, 85–87]\) (Figure 2). However, as for the evolution of nonrandom mating, the evolution of dispersal has been studied in a variety of contexts and not only in reference to the process of the reinforcement of local adaptation or speciation and a large number of factors interact to shape this trait \([88]\). However, in the context of the evolution of dispersal in presence of local adaptation there are at least two factors that cannot be ignored. The first is that, as in the case of the evolution of assortment, inbreeding depression causes a selection pressure in favor of dispersal \([89]\). This inbreeding depression can be partly, but not only, caused by the loci responsible for the local adaptation. The second factor is kin selection (Figure 3). As soon as one considers a stochastic model for the evolution of dispersal, kin selection occurs and must be taken into account to determine how dispersal evolves \([90–92]\). Intuitively, it is straightforward to see that a given allele causing zero dispersal cannot fix in a subdivided population. In other words, zero dispersal cannot be a convergent stable state as was suggested in deterministic models of reinforcement. As expected from this heuristic argument, kin selection favors more dispersal than predicted in a deterministic model \([54]\). However, this is not the only effect as local adaptation interacts with the effect of kin selection: strong differentiation at a local adaptation locus magnifies kin selection at short recombination distance. This indirect kin selection can cause bistability (i.e., different dispersal rate can evolve depending on the initial conditions), which changes qualitatively the expectation \([54]\). There are different ways to reduce dispersal, and all may not be equivalent even if the selection pressures at work will share strong similarities. In particular it is clearly important to distinguish between dispersal and habitat choice. As we have seen dispersal cannot evolve to very low rates because

![Figure 2: Indirect selection on a migration modifier with local adaptation. Sketches how a migration modifier evolves in presence of local adaptation (for the sake of illustration the M allele causes maximal migration (1/2) and the m allele zero migration). Before migration (step 1) consider two habitats with haploid individuals. Before migration (step 1) consider two habitats with haploid individuals. On the left the A allele is favored at a local adaptation locus whereas a is favored on the right. To make things simple we consider these alleles to be fixed where they are beneficial. During migration only individuals with allele M move between habitats (step (2) migrants in red). Half of the M individuals move to the other habitat and the other half stays at home. Importantly migration directly generates LD between the M allele and the locally inferior allele (the locally inferior allele is found only with M and not with m). Finally, selection occurs favoring A on the left and a on the right very strongly in the illustration and carries the m allele with the adaptation locus because of the linkage disequilibrium generated at the previous step (the m overall frequency has raised from 1/2 to 2/3 on the illustration). Note that in a finite population kin selection by contrast favors M \([54]\).](image-url)
of kin selection. However, choosing the natal habitat (to maintain local adaptation) while quitting its natal patch (to release kins from competition) may provide the best from both worlds and is therefore a more likely candidate trait for reinforcement. Two-allele models also provide several alternatives [38, 57].

3.3. Comparisons among Reinforcement Traits. The common effect in all these processes is that alleles that favor more assortment, less dispersal or tighter linkage become associated with locally beneficial alleles, which in turn generates an indirect selection pressure in their favor. In each of these cases however, the way linkage disequilibrium is built between the modifiers and the locally beneficial alleles is distinct (compare Figures 1, 2 and 4). First, a modifier has an immediate effect on the genetic composition of the population, here genotypic frequencies at the local adaptation loci: dispersal modification changes allelic frequencies; assortment changes within locus associations; recombination changes between loci associations. This immediate effect causes a frequency change at the modifier locus if there is selection on alleles, dominance, and epistasis, respectively. When the modifier changes within or between loci associations, a secondary effect occurs. Increased associations generate a higher variance in fitness, more efficient selection, and thus an increase of the frequency difference between habitats at the local adaptation locus. As a consequence, modifiers increasing these associations (modifier increasing assortment or reducing recombination in our examples) become associated, and hitchhike, with locally beneficial alleles. There

Figure 3: Kin selection on a migration modifier. Sketches how a migration modifier evolves because of kin selection. As in Figure 2, the M allele causes maximal migration (1/2) and the m allele specifies zero migration). Before migration (step 1), consider two subpopulations. In one of them the M allele is frequent (1/2), but it is absent in the other. During migration, only M individuals move between habitats (step 2), the migrant is shown in red). Half of the M individuals move to the other habitat and the other half stays at home. Then reproduction occurs (step 3). All individuals produce say, two offsprings (note that all individuals have the same survival and reproduction). Finally, population regulation occurs: juveniles compete to repopulate each subpopulation with four adults and all have the same chance to get established. After this step, the M frequency has risen to (1/3 + 1/5)/2, which is greater than 1/4, the initial frequency. There is thus selection on M allele, which is traditionally explained in terms of “kin selection”: the migrating M allele sacrifices itself by competing in a more crowded population, but it leaves room behind that benefits the other M allele, which will compete in a less crowded population. The decreased chance of survival by the migrating M (1/5–1/4) is more than compensated by the increased chance that the remaining M allele will survive (1/3–1/4). This process requires only that the M alleles are concentrated in the same population at step 1 (i.e., it requires population structure or relatedness), which is easily generated by drift [54].
are thus several ways to promote distinct genetic clusters between habitats and the three examples detailed in this paper illustrate each of these cases: directly magnifying allelic frequency differences between populations (case illustrated by dispersal modification), promoting within locus associations (case illustrated by assortment modification), promoting between loci associations (case illustrated by recombination modification). Reinforcement may occur by the evolution of many other traits than the ones mentioned here (in particular involving two-allele mechanisms, see Table 1), but their impact are likely to be achieved via one of these effects alone or in combination. Considering the three possible impacts of a modifier on the genetic composition of populations (on frequencies, within locus and between loci associations) may be a useful typology to understand the different ways reinforcement and genetic clustering can occur. It is orthogonal to, and complements the usual one-versus two-allele classification (Table 1).

4. Conclusion

The first conclusion is that the process of reinforcement and local adaptation are intertwined and occur simultaneously. Whether pre- and postzygotic isolation will eventually evolve is uncertain in such a dynamic process. In particular, local adaptation can collapse if generalist alleles arise and spread. However, there are several positive feedback loops that will tend to drive the system towards divergence (the reinforcement, demographic, and recombination loops).
From a theoretical standpoint, this process has rarely been analyzed jointly and in a dynamic way with changes in local adaptation itself. In the context of mounting evidence in favor of ecological speciation [106, 107], such an approach would certainly help evaluate its likelihood, tempo, and mechanism.

Second, Felsenstein [21] proposed to distinguish the different mechanisms for reinforcement on the idea that they involved the spread of one or two-alleles in the incipient species. This distinction is an important one, but it is not the only one to be made. Many one-allele mechanisms are only superficially similar as they can promote genetic clustering and speciation in different ways. A useful typology could be that they increase differentiation among populations, heterozygote deficit, or linkage disequilibrium, which corresponds to modifying one of the three fundamental events in a sexual life cycle (dispersal, syngamy, or meiosis, resp.). Furthermore, different traits may increase genetic clustering, but may not contribute to reinforcement because they are exposed to a variety of other selective effects. Models of reinforcement based on the evolution of particular traits must integrate what is known outside the speciation literature for those traits. For instance, recombination [108], mating systems [109], and dispersal [110], as discussed above, have all been intensely studied outside this context pinpointing a variety of selective effects. These theoretical developments certainly have to be merged.

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References


Research Article

Testing the Role of Habitat Isolation among Ecologically Divergent Gall Wasp Populations

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Habitat isolation occurs when habitat preferences lower the probability of mating between individuals associated with differing habitats. While a potential barrier to gene flow during ecological speciation, the effect of habitat isolation on reproductive isolation has rarely been directly tested. Herein, we first estimated habitat preference for each of six populations of the gall wasp Belonocnema treatae inhabiting either Quercus virginiana or Q. geminata. We then estimated the importance of habitat isolation in generating reproductive isolation between B. treatae populations that were host specific to either Q. virginiana or Q. geminata by measuring mate preference in the presence and absence of the respective host plants. All populations exhibited host preference for their native plant, and assortative mating increased significantly in the presence of the respective host plants. This host-plant-mediated assortative mating demonstrates that habitat isolation likely plays an important role in promoting reproductive isolation among populations of this host-specific gall former.

1. Introduction

Ecological speciation describes the process by which reproductive isolation evolves as a consequence of divergent natural selection between environments [1, 2]. Studies of ecological speciation seek to associate the origin of specific reproductive isolating barriers that reduce gene flow with sources of divergent selection [3]. Throughout the modern synthesis, biologists described a central role of ecological adaptation in the speciation process [4–6]; however, it was not until a recent renaissance of empirical study that specific ecological barriers have been experimentally shown to contribute to reproductive isolation. In strong support of the role of ecology in speciation, a comparative study across many plant and animal taxa provided evidence that ecological adaptation generally contributes to the evolution of reproductive isolation [7].

Recent studies of the role of ecology in speciation have documented a central role of divergent natural selection in the speciation process among a diverse set of taxa (e.g., Rhagoletis fruit flies [8], Littorina snails [9], Neochlamisus leaf beetles [10], Gasterosteus aculeatus sticklebacks [11], Gambusia fishes [12], Timema walking sticks [13], Mimulus monkeyflowers [14], and cynipid gall wasps [15]). Moreover, these studies have documented that a diversity of both prezygotic and postzygotic reproductive barriers can arise as a result of divergent ecological adaptation [3, 16], including temporal isolation [17], sexual isolation [18], cryptic isolation [19], and extrinsic (ecological) postzygotic isolation [20, 21].

The study of ecological speciation has been especially informed by studies of herbivorous insects [22, 23]. The intimate interactions between herbivorous insects and their host plants suggest a strong role for divergent natural selection in promoting diversification. Herbivorous insects tend to be highly specialized in their use of host plant taxa [24], and specialized insect herbivores can exhibit pronounced geographic variation in, and rapid evolution of, host plant preference and performance traits (e.g., [25, 26]). The increased rates of speciation associated with herbivory among insects
provides evidence that host plant ecology may generally contribute to the speciation process [27, 28].

Walsh [29] was one of the first to associate phenotypic variation among insects with the host plants upon which they were found and Bush [30] was one of the first to argue for a direct role of host-plant-associated selection in the genesis of new insect species. Continued work has since highlighted the role of divergent selection due to host plant use among taxa where gene flow is possible (e.g., [8, 10, 13, 17, 31, 32]). A critical barrier to gene flow among specialist herbivore insect taxa is “habitat isolation” [8, 16, 30]. Habitat isolation for host-specific phytophagous insect species describes the process by which the differing habitat preferences of insect populations associated with alternative host plants reduces the frequency of encounters and thus the likelihood of mating between individuals from the differing host-associated populations. For example, Nosil et al. [33] examined 27 populations of *Timema cristinae* walking sticks feeding on *Ceanothus* or *Adenostoma* host plants. Populations of walking sticks on different host plants expressed stronger divergence in host plant preference than populations on the same host plant. These differences likely result in reduced encounters among individuals preferring different hosts. Similar inferences regarding the role of host plant preference in speciation have been made for leaf beetles [10], pea aphids [32], ladybird beetles [34], *Rhagoletis* fruit flies [8, 35], and *Eurosta* galling flies [36].

However, rarely has the effect of observed differences in host plant preference on reproductive isolation been tested directly [23]. Field studies of the apple and hawthorn host races of *Rhagoletis pomonella* found evidence that host plant preference could generate habitat isolation [8]. Here, the apple and hawthorn host races returned to their natal plant species when released in the presence of both apple and hawthorn trees. Because these host races mate on their host plant it is likely that host preference translates into host-associated assortative mating that restricts gene flow between the ecologically divergent populations. In a direct laboratory-based test of habitat isolation, Funk [10] performed mating assays among ecologically divergent host forms of the leaf beetle *Neochlamisus bebbianae*. To isolate the role of the host plant on overall sexual isolation, the host plant of each individual was included in half of the mating assays. Results from Funk [10] were mixed, with one of the four different host comparisons of *N. bebbianae* populations exhibiting a significant increase in assortative mating due to host plant presence.

In the present study, we use a combination of habitat preference and mate preference assays among ecologically divergent populations of the gall wasp *Belonocnema treatae* (Hymenoptera: Cynipidae) to test for (a) variation among host-associated populations in habitat (i.e., host plant) preference and (b) an explicit role for habitat isolation in overall reproductive isolation. We test these hypotheses using populations of *B. treatae* that inhabit two sister species of live oak, *Quercus virginiana* and *Q. geminata*, which geographically overlap in the southeastern United States. The habitat of each oak differs slightly, with *Q. virginiana* occurring in moister, more nutrient rich, and higher pH sites than *Q. geminata* [37], and the oaks themselves differ in leaf morphology and flowering times [38]. Populations of *B. treatae* that inhabit these oak species exhibit significant differences in root gall structure and adult body size that are associated with host use, and gall wasp populations exhibit host-associated assortative mating [15].

### 2. Methods

#### 2.1. Study System and Sampling

*Belonocnema treatae* is a host-specific gall former [39] that exhibits regional specificity (Ott and Egan, personal observation) on species of live oak, *Quercus*, within the *Virentes* series of the genus [40]. *Belonocnema treatae* exhibits a heterogenous life cycle with temporally segregated sexual and asexual generations [39]. The asexual generation develops within single-chambered, spherical galls on the undersides of leaves during the summer and fall and emerges in the winter. The sexual generation develops within multichambered galls on the root tissue, and males and females emerge during the spring. We collected root galls containing the sexual generation from six allopatric populations in central Florida in April 2010 (three *Q. virginiana* and three *Q. geminata* populations; see Table 1 for location information). Galls were husbanded under common laboratory conditions (12:12 light : dark, 23°C), and upon emergence adults were sorted by sex and population for host preference and mating assays, which took place within 48 hours of emergence.

#### 2.2. Host Preference Assays

Trials took place within 25 × 8 cm clear-plastic cups stocked with a cutting of each host

<table>
<thead>
<tr>
<th>Population</th>
<th>Host association</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near Avon Park (AP)</td>
<td><em>Q. geminata</em></td>
<td>27° 36' 00'' N</td>
<td>81° 30' 42'' W</td>
</tr>
<tr>
<td>Scrub field (S)</td>
<td><em>Q. geminata</em></td>
<td>27° 30' 48'' N</td>
<td>81° 20' 16'' W</td>
</tr>
<tr>
<td>Archbold Biological Station (ABS)*</td>
<td><em>Q. geminata</em></td>
<td>27° 10' 57'' N</td>
<td>81° 21' 08'' W</td>
</tr>
<tr>
<td>Near Hickory Hammock Natural Area (HH)*</td>
<td><em>Q. virginiana</em></td>
<td>27° 24' 09'' N</td>
<td>81° 06' 42'' W</td>
</tr>
<tr>
<td>Gatorama (GR)</td>
<td><em>Q. virginiana</em></td>
<td>26° 55' 30'' N</td>
<td>81° 18' 44'' W</td>
</tr>
<tr>
<td>Near Koreshan State Park (KSP)</td>
<td><em>Q. virginiana</em></td>
<td>26° 26' 04'' N</td>
<td>81° 48' 56'' W</td>
</tr>
</tbody>
</table>

* Denotes populations used in the mate preference tests.
plant (Q. virginiana and Q. geminata). A single B. treatae was aspirated into each cup and then observed at five-minute intervals for one hour for a total of 12 observations. At each time point, we recorded the location (on Q. virginiana, on Q. geminata, or on the cup) of each individual. Both sexes were tested. Host preference was calculated for each individual as the relative time spent on one host plant species divided by the total time spent on both host plants during the trials (e.g., individual preference for Q. virginiana = (number of observations on Q. virginiana)/(number of observations on Q. virginiana + number of observations on Q. geminata)). We performed a total of 214 preference assays distributed across the six B. treatae populations (see Figure 1 for sample sizes per population).

2.3. Assays of Sexual Isolation with an Explicit Test of Habitat Isolation. No-choice mating trials were conducted to test for assortative mating as a function of population of origin between one population (ABS—Archbold Biological Station, FL) of B. treatae reared from galls that developed on Q. geminata and one population (HH—near Hickory Hammock State Natural Area, FL) reared from galls on Q. virginiana (N = 291 total; see Figure 2 for sample sizes per treatment). Trials again took place within 25 × 8 cm clear-plastic cups. In half of the trials we placed a small, defoliated, dried twig for the wasps to walk on as a control. Alternatively, in half the trials a small leaf-bearing section of stem of the species of oak representing each individual’s host plant was added. One male and one female were then aspirated into each cup (replicate). Each pair was observed at

\[
\text{Host preference of individual } B. \text{ treatae } \text{gall wasps expressed for each of three } Q. \text{ virginiana and three } Q. \text{ geminata host-associated populations during choice tests that paired each wasp's native/natal host with the alternative oak species. Illustrated is the mean (±SE) of the proportion of time spent on the native host plant by each sex within each population. The dashed line highlights no preference (defined as 50% of time spent on each host). Note each population differed significantly from 50% (P < 0.0001). Numbers above the SEs are the number of replicates. Note the reversed left and right y-axis.}
\]
five-minute intervals for one hour for a total of 12 observations. At each survey we recorded each individual’s location (on Q. virginiana, on Q. geminata, or test arena) and whether the pair was copulating. Copulations were defined as males having mounted the female with abdomens in contact. An additional estimate of host plant preference was calculated during these mating trials based on the proportion of time (n/12 observation periods) that wasps of each sex were observed on each host plant. Estimates were then converted to a relative value of host preference as previously described. For interpopulation pairings, the average host preference of males and females was compared to the probability of a successful hybrid mating (i.e., copulation).

2.4. Statistical Analyses. To test for differences in host plant preferences of individual wasps between the sexes, among populations, and their interaction, we conducted an ANOVA on individual relative preference for Q. virginiana (1-preference for Q. geminata) followed by Tukey’s HSD test to compare means among populations. Population was treated as a random effect; sex was treated as a fixed effect. A similar analysis of host preference expressed by individual wasps from the two population sources when the sexes were paired for the mating assays was also performed. We also compared each population’s relative preference for its native host to a value of 0.5 by means of a $t$-test. The value 0.5 indicates equal time spent on each of the two host plants and characterizes “no preference.”

To test for assortative mating, we used logistic regression to examine the effects of male host plant, female host plant, the presence/absence of the host plant, and their interactions on copulation frequency in the mating assays. The two-way interaction term, female host plant × male host plant, tests for overall assortative mating whereas the three-way interaction term, female host plant × male host plant × host plant present/absent, tests the effect of habitat preference on assortative mating. To examine the role of habitat isolation on sexual isolation further, we compared the host preference expressed by the male and female in each interpopulation mating assay when host plants were present between those assays that resulted in a “hybrid” copulation and those that did not by means of a standard $t$-test.

3. Results

3.1. Habitat Plant Preference. Geographic variation in relative host plant preference among the six B. treatae populations is evidenced by the significant population term in the ANOVA of host preference assays (Table 2). The difference in preferences among the populations is clearly associated with the host plant from which the B. treatae populations were collected (Figure 1). Each population preferred its native host, as shown by the highly significant difference between relative preference for native host and the no-choice expectation of equal time ($t$-test of population mean versus 0.5; KSP $t_{df=32} = 6.31$, HH $t_{df=41} = 6.76$, GR $t_{df=36} = 5.42$, AP $t_{df=29} = 8.11$, ABS $t_{df=38} = 5.45$, S $t_{df=33} = 10.11$; $P < 0.0001$ for all comparisons). There was also significant variation among Q. virginiana associated populations in the degree of preference for the native host plant. The KSP and HH populations exhibited stronger preferences than the GR population as shown by Tukey’s HSD test following the ANOVA (KSP = 0.71 ± 0.02SE, HH = 0.70 ± 0.02SE, GR = 0.60 ± 0.02SE; Tukey’s HSD test: $[KSP = HH] > GR$, $P < 0.05$; Figure 1). The three populations of B. treatae associated with Q. geminata did not differ in their degree of native host preference (ABS = 0.33 ± 0.02SE, S = 0.28 ± 0.03SE, AP = 0.29 ± 0.03SE; Tukey’s HSD test: $ABS = S = AP$; $P > 0.05$; Figure 1). Females consistently expressed stronger host preference than did males for their native host plant across all six populations (Figure 1). The significant population × sex interaction term in the ANOVA (Table 2) demonstrates that the degree of difference between males and females in host preference varied by population. For example, males and females from KSP preferred their native host Q. virginiana similarly, but males and females from HH varied by over 30%, with females expressing strong host preference for Q. virginiana and males spending a similar amount of time on each host plant (Figure 1).

3.2. Host Plant Effects on Mating Preference. Patterns of B. treatae copulation frequency (number of copulations/number of mating trials) revealed strong evidence of host-associated sexual isolation between individual gall wasps from the HH Q. virginiana and the ABS Q. geminata source populations. Importantly for the hypothesis that habitat isolation drives reproductive isolation, B. treatae gall wasps were more likely to copulate when paired with individuals from the same host plant than from the alternative host plant as shown by the significant interaction term, female host × male host (Table 3, Figure 2). This result is explained in part by the 47% decrease in the frequency of between-host matings when host plants were present during the mating assays (Figure 2). This result is likely due to the intrinsic effect of habitat isolation arising from host preference that is effective even within the confines of the small enclosures used for the mating assays.

The average host preference expressed by paired male and female B. treatae during mating trials was similar to that revealed by the testing of individuals shown in Figure 1. However, if a mating did occur between B. treatae from different host plants, the average host preference expressed by a pair of individuals was associated with the degree of sexual isolation among them. Interestingly, this appeared to

<p>| Table 2: ANOVA: sources of variation in relative host plant preference of individual B. treatae assessed from no-choice preference assays. |</p>
<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>5</td>
<td>2124.1</td>
<td>29.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>10.1</td>
<td>0.71</td>
<td>0.4009</td>
</tr>
<tr>
<td>Population × sex</td>
<td>5</td>
<td>585.7</td>
<td>8.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>203</td>
<td>5803.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
be driven by variation of the male preference (t-test: $t = 3.875$, df = 62, $P < 0.0003$; Figure 3(A)) rather than the female preference (t-test: $t = -0.49$, df = 63, $P = 0.9614$; Figure 3(B)).

4. Discussion

4.1. Host Preference, Mating Preference, and Population Differentiation. Spatially divergent selection among populations leading to local adaptation is recognized as being central to initiating the divergence of incipient species [41]. However, dispersal and gene flow among populations experiencing differing selective regimes is antagonistic to local adaptation, population differentiation, and speciation [42]. Habitat isolation arising from the evolution of habitat preferences can reduce dispersal between contrasting habitats and promote adaptive divergence [8, 10, 33, 36, 43]. Perhaps nowhere is this more evident than among herbivorous insect specialists, who tend to oviposit, feed, rest, develop, and mate on their host plants [23]. In the present study, we demonstrated that multiple populations of the gall former *B. treatae*, each inhabiting either of two closely related oak species [37, 38] each express strong preferences for their natal host plant species. This preference was especially apparent among females as shown in Figures 1 and 3. Our results are consistent with partial habitat isolation in gall wasps evolving as a byproduct of adaptation to different hosts, as has now been demonstrated in a number of plant-insect systems [8, 10, 20]. Our experimental assays of host preference demonstrated that (a) each host-associated population accepts its native oak more than the alternative sister species of oak, (b) populations vary in the degree of host preference exhibited, and (c) individuals within host-associated populations vary in the strength of their preference, a proxy for ecological specialization. In light of the spatial distribution of the two species of oaks throughout central Florida, our results suggest an active role for habitat isolation in the ongoing evolution of reproductive isolation in this species of gall former. Given that the expansive geographic range of *B. treatae* (Florida to Texas) spans the geographic ranges of the six species of closely related live oaks that constitute the series *Virentes* [37, 38, 40], our results hint at the as yet unexplored prospect of replicated regional differentiation in host preference within *B. treatae*. 
An underlying assumption of our current measurement of host preference is that time spent on a host plant is correlated with mating and oviposition decisions, as has been shown in other host-associated and ecologically divergent insect populations [44]. However, we consider residence time in our study to be a conservative measure of host preference, as in two other species that form host-associated populations, *E. solidaginis* and *P. pomonella*, the insect is more likely to sit on the alternate host plant than to mate or oviposit on it [8, 44].

Through mating assays we showed that individual *B. treatae* prefer to mate with individuals from their natal oak population rather than individuals from the alternative oak species. Most important to the present study was our explicit test of the role of host preference, an estimate of habitat isolation, on the degree of sexual isolation among these ecologically divergent host-associated populations. Here, comparing sexual isolation with and without host plants being present in the experimental arena does just this [10, 23]. We found that the presence of the host plant during mating trials increased the degree of sexual isolation among *B. treatae* reared from different host plants by reducing between-host matings by 47%. Moreover, we found this effect to be associated with variation in the host plant preference exhibited by males as most matings occurred on the female’s host plant, regardless of the type of cross (interpopulation or intrapopulation).

Our mating assays constituted “no-choice” conditions where females were paired with males from one of two possible populations. An alternative approach can involve “choice” experiments, in which individuals can choose between inhabitants from each population. “Choice” tests can offer a different perspective of mate choice, commonly observing stronger preferences than “no-choice” tests [45]. In our assays, we imagined the biologically relevant scenario to be one in which an individual on its native host plant encountered a single migrant in a “no-choice” type scenario.

All adults assayed in both the host and mate preference trials were reared directly from their native host hence we cannot rule out host environment as a contributing factor to the observed patterns of host and mate preferences. Reciprocal transplant experiments, repeated across populations, will be needed to distinguish genetic and environmental contributions to the observed differences in habitat and mating preferences and assess the adaptive nature of these traits through measurements of the fitness of each population on the two host species. However, the observed differences in host preference, even if due to host environment, would still be expected to contribute to divergence of *B. treatae* populations, as parental generation migrant wasps would be predicted to be averse to settling on, or mating with individuals from, the alternative host plant. Thus, our experiments support a critical role for host plant use in promoting reproductive isolation among populations of *B. treatae* regardless of the underlying basis for preference variation. Future work will test additional populations in mate choice assays to assess the generality of the current support for the hypothesis that habitat isolation directly contributes to sexual isolation during mate choice.

4.2. Habitat Preference and Speciation. Divergent habitat (e.g., host plant) preferences can promote the speciation process in two ways: (a) directly by reducing encounters between potential mates and driving assortative mating and (b) indirectly by generally reducing gene flow, which facilitates overall adaptive divergence and increases the opportunity for postzygotic barriers to arise. Habitat preference is considered to act directly as a form of reproductive isolation; however, to date, only a modest number of studies have actually demonstrated that habitat preference results in assortative mating and reduced gene flow. Cage experiments show increased assortative mating between host-associated populations of *Eurosta solidaginis* gall flies when host plants are present relative to when they are absent [36]. Mark-recapture studies of hawthorn and apple host races of *Rhagoletis pomonella* flies suggest that the tendency of flies to reproduce on the same host species used in earlier life cycle stages strongly reduces gene flow between the races [8, 10]. Most convincingly, a combination of field and molecular data indicates that variation in host plant choice reduces gene flow between clover- and alfalfa-adapted populations of *Acyrthosiphon pisum* pea aphids [32].

Habitat preferences can also indirectly contribute to the speciation process by (a) reducing the constraining effects of gene flow on adaptive divergence in ecologically important traits [13, 33], (b) promoting postzygotic isolation through less fit hybrids [46–48], and (c) increasing the opportunity for Dobzhansky-Muller postzygotic barriers to arise [49]. Thus, when divergent habitat preference acts as a barrier to gene flow, additional prezygotic and postzygotic reproductive barriers can evolve via the byproduct model of ecological speciation [1, 10, 23, 50]. Under this model, reproductive barriers evolve as an indirect consequence of reduced gene flow rather than as a direct result of selection.

4.3. Conclusions. While habitat isolation is thought to play a critical role in pre-mating reproductive isolation among herbivorous insect populations and, in general, among all taxa undergoing ecological divergence and speciation, very rarely is the role of habitat selection directly tested [23]. In the present study, we used a combination of habitat preference and mate preference assays among ecologically divergent populations of the gall wasp *Belonocnema treatae* to document variation among populations in habitat (i.e., host plant) preference and examine the role of habitat isolation to overall reproductive isolation. Overall, all populations examined showed habitat fidelity and habitat preference decreased the probability of mating between individuals from alternative host plants. The increase in the degree of assortative mating due to the presence of the host plant during mate choice provides an example of the importance of habitat isolation in promoting reproductive isolation between host-plant-associated populations of herbivorous insects.

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References


Review Article

Underappreciated Consequences of Phenotypic Plasticity for Ecological Speciation

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Phenotypic plasticity was once seen primarily as a constraint on adaptive evolution or merely a nuisance by geneticists. However, some biologists promote plasticity as a source of novelty and a factor in evolution on par with mutation, drift, gene flow, and selection. These claims are controversial and largely untested, but progress has been made on more modest questions about effects of plasticity on local adaptation (the first component of ecological speciation). Adaptive phenotypic plasticity can be a buffer against divergent selection. It can also facilitate colonization of new niches and rapid divergent evolution. The influence of non-adaptive plasticity has been underappreciated. Non-adaptive plasticity, too can interact with selection to promote or inhibit genetic differentiation. Finally, phenotypic plasticity of reproductive characters might directly influence evolution of reproductive isolation (the second component of ecological speciation). Plasticity can cause assortative mating, but its influence on gene flow ultimately depends on maintenance of environmental similarity between parents and offspring. Examples of plasticity influencing mating and habitat choice suggest that this, too, might be an underappreciated factor in speciation. Plasticity is an important consideration for studies of speciation in nature, and this topic promises fertile ground for integrating developmental biology with ecology and evolution.

1. Introduction

Phenotypic plasticity has often been seen primarily as an alternative to genetic divergence and a feature making populations less responsive to natural selection [1–3]. For example, those studying adaptation and speciation have often used phrases like “merely plastic” to contrast environmentally induced variation against geographic or species differences with strong genetic bases [4–8]. However, others suggested that phenotypically plastic traits can promote adaptive evolution and the origin of species [9–16]. The general issue of plasticity and adaptation has been reviewed extensively in the last decade (e.g., [15, 17–26]). Adaptive plasticity’s impact on speciation was recently reviewed by Pfennig et al. [27], and I do not attempt to duplicate their efforts. Instead I make a few points that have not been emphasized in the recent literature. In particular, nonadaptive plasticity and environmentally induced barriers to gene flow deserve greater attention.

After making explicit my working definitions of key terms, I argue that the “developmental plasticity hypothesis of speciation” [13–15] is a special case of ecological speciation, and I review the subject by breaking down the effects of plasticity on the two components of ecological speciation: adaptive divergence and the evolution of reproductive isolation [28]. I close with a few suggestions for future work.

By any definition, speciation requires genetic divergence. Therefore, integration of ecological developmental biology with the well-developed body of fact and theory on the genetics of speciation [29–31] will be more productive than attempting to replace this population genetic foundation. Recent reviews and models support this perspective [27, 32–37].

2. Definitions

Understanding the relationship between environmental induction and speciation requires a set of consistent definitions. Terms like “environment,” “speciation,” “plasticity,” and “natural selection” are sometimes assumed by
Adaptation. Genetic change in response to natural selection and resulting in organisms with improved performance with respect to some function or feature of the environment.

Countergradient Variation. Pattern of geographic variation in which genetic differences between populations affect their phenotypes in the opposite way from environmental differences between populations. For example, if the mean phenotype of population 1 is given by the sum of genetic and environmental effects \( P_1 = G_1 + E_1 \), and the effect of environment 2 tends to increase the phenotypic value relative to environment 1 \( (E_2 > E_1) \), countergradient variation would exist if \( G_2 < G_1 \). Without genetic differentiation the expected difference in phenotype would be \( P_2 - P_1 = E_2 - E_1 \), but countergradient variation reduces that difference and might even make \( P_2 < P_1 \). More formally, countergradient variation is negative covariance between genetic and environmental effects on phenotype [41].

Environment. Here, I consider environment to include anything external to a given individual organism. Environment includes other organisms (siblings, mates, competitors, predators, prey, etc.) in addition to the physical and chemical surroundings. Given this definition, different individuals in the same place might experience different environments. Neither ecological speciation nor environmentally induced variation require environmental differences to be associated with geography. To put it another way, the effects of environment on fitness and on development might differ among co-occurring individuals for a variety of reasons, often involving feedbacks between phenotype and environment [42]. For example, small tadpoles in a pond might experience food shortages or attacks from predators while large tadpoles in the same pond have access to more food and experience less predation (or a different set of predators).

Genetic Assimilation. Evolutionary reduction in the degree of plasticity such that a character state or trait value that was once conditionally expressed depending on the environment becomes expressed constitutively (unconditionally, regardless of environment).

Natural Selection. Natural selection refers to any nonrandom difference between entities in survival or reproduction. To put it another way, natural selection exists whenever phenotypic variation causes covariance between phenotype and fitness [43, 44]. Fitness is metaphorical shorthand for the ability to survive and reproduce. It is important to emphasize that natural selection, under this definition, can exist without genetic variation and can recur over many generations without causing evolution [15, 17].

Plasticity. The ability of a single genotype to express different phenotypic values or states under different environmental conditions, that is, in response to environmental induction. Plasticity can include developmental plasticity, physiological acclimation, or behavioral flexibility. Plasticity might be adaptive or not. Adaptive plasticity is a tendency for a genotype to express a phenotype that enhances its ability to survive and reproduce in each environment. Nonadaptive plasticity includes any response to environmental induction that does not enhance fitness (including maladaptive responses). Noisy plasticity is effectively unpredictable phenotypic variation owing, for example, to developmental instability or random perturbations within environments [45]. Phenotypic plasticity and environmental induction are twin concepts; plasticity emphasizes an organismal property (the propensity to express different phenotypes in different environments), and environmental induction emphasizes the action of the environment. Phenotypic plasticity might exist even in a homogeneous population in a homogeneous environment. Environmental induction happens when environmental heterogeneity causes phenotypic heterogeneity.

Polyphenism. Expression of more than one discrete phenotypic state (alternative phenotypes) by a single genotype (a special case of phenotypic plasticity).

Reaction Norm [or Norm of Reaction]. The set of expected phenotypic states or values expressed by a genotype over a range of environments.

Speciation. Speciation is any process in which an ancestral species gives rise to two or more distinct descendant species. There is some disagreement about whether “speciation” should be synonymous with the evolution of reproductive isolation [30, 46, 47] or broadened to include anagenesis or phyletic speciation [48–50]. In any case, speciation is usually a gradual, continuous process of genetic divergence resulting in a discontinuous pattern of variation (species taxa). Ecological speciation is the evolution of reproductive isolation as a consequence of divergent ecological adaptation [28, 51, 52]. Without reproductive isolation, this is local adaptation. This definition is based on the biological species concept [53], which emphasizes genetically based reproductive isolation as the primary explanation for the existence of distinct kinds of organisms (i.e., those recognized as species taxa). I am not making a recommendation about taxonomic practice. Rather, from the perspective of evolutionary biology, the evolution of reproductive isolation is what distinguishes speciation from more general phenomena of genetic divergence [30, 40].
3. The Developmental Plasticity Hypothesis of Speciation

Matsuda [54] hypothesized that phenotypic plasticity was a crucial first step in the adaptive evolution of distinct, ecologically specialized lineages. As an example, Matsuda [54] suggested that major differences in life history, such as presence or absence of metamorphosis prior to reproduction in salamanders (Figure 1), likely began as nongenetic polyphenisms and evolved via genetic assimilation in habitat specialists. Widespread generalists such as *Ambystoma tigrinum* and *A. velasci* show conditional expression of metamorphosis from aquatic larva to terrestrial adult in small temporary ponds versus a fully aquatic life cycle with no metamorphosis in more permanent water bodies. This polyphenism likely represents the ancestral state of the tiger salamander clade [55]. In several isolated lakes in Mexico, permanently aquatic endemics such as *A. mexicanum* and *A. dumerili* no longer express metamorphosis in nature owing to genetic changes in the thyroid hormone system [54–56].

Along the same lines, West-Eberhard [13–15] proposed a generalized "developmental plasticity hypothesis of speciation" in which the evolution of ecologically distinct forms in different environments depends on the initial appearance of those distinct forms as alternative phenotypes in a phenotypically plastic ancestor. She argued that when adaptive phenotypic plasticity results in strong associations between phenotypes and environments, rapid speciation could occur in three steps. First, alternative phenotypes become fixed in different populations owing to environmental differences, but with little or no genetic change. Then, genetic assimilation and/or other adaptive modifications of each phenotype occur owing to divergent selection. Finally, reproductive isolation evolves as a byproduct of adaptive divergence or via reinforcement if there is contact between the diverging populations.

Clearly, West-Eberhard’s [13–15] hypothesis is a kind of ecological speciation in which developmental plasticity promotes genetic divergence in response to ecologically based selection. In the absence of plasticity, divergence might be prevented entirely if the single expressed phenotype cannot establish a viable population in the alternative environment [22, 27, 36] or might be much slower if phenotypic divergence must await new mutations and their gradual fixation [13–15].

West-Eberhard’s developmental plasticity hypothesis of speciation is focused on adaptive phenotypic plasticity and its influence on one component of ecological speciation: the evolutionary response to divergent selection. However, nonadaptive plasticity might be equally or more influential in promoting an evolutionary response [18]. Further, the other component of ecological speciation, the evolution of reproductive isolation [28, 62, 63], also can be directly influenced by phenotypic plasticity. In the next sections, I examine how plasticity can interact with these two components of ecological speciation.

![Figure 1: The tiger salamander radiation exemplifies](image)

4. Phenotypic Plasticity and Ecological Divergence

Phenotypic plasticity can slow or enhance genetic divergence. How plasticity affects divergence depends to some extent on whether plasticity is adaptive or not.

4.1. Adaptive Plasticity. Adaptive plasticity can dampen or eliminate divergent selection. If any individual can express a nearly optimal phenotype in whatever environment it finds itself, then there is little or no variation in the ability to survive and reproduce, hence little or no divergent selection [64]. This has long been an intuitive reason to regard plasticity as a constraint on genetic evolution and to discount the evolutionary potential of environmentally induced variation [1]. Models have supported the prediction that adaptive plasticity can effectively take the place of genetic divergence between environments [65–67]. And a large number of empirical studies are consistent with increased plasticity in species with high dispersal rates [68]. However, the extent to which plasticity prevents or slows genetic divergence depends on several factors explored by Thibert-Plante and Hendry [36] in individual-based simulations.

First, is development sufficiently flexible that an individual can express traits near either environmental optimum? Given alternative environments or niches with divergent fitness functions, the only way environmental induction can completely eliminate divergent selection is to cause the mean expressed trait of a single gene pool to match the optimum in each environment [22] (Figure 2). If adaptive plasticity is less than perfect, divergent selection might still exist. Then the question is whether plasticity quantitatively dampens...
the fitness tradeoff enough to substantially slow or prevent
divergent evolution [36].

Second, how much dispersal occurs between environ-
ments? In the absence of gene flow, almost any amount
of divergent selection will eventually cause evolutionary
divergence. When there is gene flow between populations,
the effect of divergent selection depends on the relative
magnitudes of selection and gene flow [1, 69–73], in addition
to demographic factors [74–76]. Therefore the impact of
adaptive plasticity on the potential for genetic divergence
depends on how it affects the tension between divergent
selection and gene flow [36, 77, 78]. Moreover, plasticity itself
is adaptive only to the extent that individuals have a reason-
able chance of experiencing alternative environments. If the
populations expressing alternative phenotypes are isolated
in their respective environments, the ability to express the
alternative phenotype is likely to be lost owing to selection
for efficient development or simply because loss of function
mutations are likely to accumulate neutrally in genes that
are never expressed [79]. This process of a conditionally
expressed trait becoming constitutively expressed is genetic
assimilation [11, 16, 80].

Third, are the systems sensing the environment and
regulating trait expression sufficiently accurate that the
best phenotype is reliably expressed in each environmental
context? There are two components to this, first is simply the
question of how well developmental or behavioral systems
are able to sense and react to environmental stimuli [81].
Again, if adaptive plasticity is less than perfect, divergent
selection can exist. Second is the question of whether the tim-
ing of key developmental and life history events is such that
future environmental conditions can be correctly predicted
[36]. For example, if individuals disperse and settle before
completing development (e.g., seeds or planktonic larvae),
they might be able to accurately tune their adult phenotypes
to the environment in which they settle. However, if a
substantial number of individuals disperse after completing
development (e.g., animals with extended parental care
[82]), then developmental plasticity would do little to help
them accommodate new environmental challenges because
their phenotypes are adjusted to their natal habitat rather
than their new habitat. In this case, there might be strong
selection against immigrants before any genetic differences
arise between populations [36].

Finally, is there any cost to plasticity? Several modeling
studies have confirmed the idea that plasticity is less likely
to evolve if there are fitness costs to maintaining multiple
developmental pathways or changing expression during
development [36]. Empirical tests for costs of plasticity itself
are rare [83], but it is conceivable that some pathways might
have inherent tradeoffs between efficiency and plasticity
[84, 85], and adaptive plasticity probably always comes with
some potential for error; that is, the best developmental
“decision” might not be made every time [64, 81]. When
plasticity is costly enough to outweigh its fitness benefits,
possible alternative outcomes are the evolution of a single
“compromise” or generalist phenotype, evolution of a simple
genetic “switch” enabling coexistence of alternative specialist
phenotypes [14, 15, 86], or divergent evolution of specialist
populations (local adaptation) [45, 67, 87]. In general we
know very little about the prevalence or influence of costs
of plasticity in nature.

The potential for adaptive plasticity to evolve as a re-
sponse to ecological tradeoffs instead of genetic divergence
is well supported. The dampening effect of plasticity is reduced
but not eliminated by reduction in the extent and precision
of plasticity, reduction in gene flow between environments,

![Figure 2: Effects of adaptive plasticity on colonization and adaptation to a new niche. A population well adapted to its niche is illustrated in (a) by coincidence of the mean trait value (black line) with the peak of the fitness function (dashed line). If the fitness function is dramatically different in a new environment, a population with trait values favored in the old environment (b) might have such low fitness as to have little chance of survival. If environmental induction produces a shift in trait values toward higher fitness phenotypes (c), the population might persist but still experience selection. If phenotypic plasticity results in a perfect match between mean trait value and fitness optimum (d), then there is no effect of selection on the population mean.](image)
Gene expression

Temperature

(a)

(b)

Figure 3: Adaptive and nonadaptive reaction norms. These hypothetical examples suppose that the optimum expression level for some gene increases with temperature and that increased temperature induces increased expression of the gene. (a) Plasticity is adaptive when it keeps expression closer to the optimum than it would be if expression were constant across temperatures. (b) Plasticity is nonadaptive (maladaptive, in this case) when induced changes in a new environment take expression further from the optimum than it would be if it had remained constant. (b) is an example of countergradient variation, in which genetic differences cause cool-adapted genotypes to have higher gene expression than warm-adapted genotypes at the same temperature. The result is overexpression by cool-adapted genotypes transplanted to warm environments.

and increased costs of plasticity [36]. However, plasticity can actually promote genetic divergence under some conditions. In particular, when development is completed after dispersal (e.g., sessile organisms), adaptive plasticity might make successful colonization of new environments more likely (Figure 2). In West-Eberhard’s [14, 15] conceptual model and Thibert-Plante and Hendry’s [36] mathematical model, individuals are able to colonize a radically new environment by adjusting developmentally, behaviorally, and/or physiologically. This adaptive plasticity allows a population to persist in the new environment, continually exposed to divergent selection. If instead all individuals entering the new environment die or leave, there is no divergent selection. Thus, without adaptive plasticity, there might simply be suitable and unsuitable environments, with little opportunity for divergent evolution. Successful colonization of a new environment can initiate divergent selection, not only on the plastic trait, but possibly also on other traits, which might then cause ecological speciation. This to some extent reconciles the conflicting effects of plasticity. In other circumstances, when dispersal occurs after development (e.g., animals with extended parental care), individuals settling in new environments are especially likely to express suboptimal phenotypes, which might accentuate the effects of selection and spatial separation once a new habitat has been colonized [36]. This effect is similar to effects of nonadaptive plasticity discussed below.

4.2. Nonadaptive Plasticity. The potential for adaptive plasticity to promote colonization and adaptation to alternative environments has been promoted by advocates of developmental evolutionary biology [14, 15, 17, 88] and treated extensively in recent reviews and models [22, 27, 32, 36]. The effects of nonadaptive phenotypic plasticity have received less attention. However, any environmental effect on phenotypes can affect the strength and direction of selection in addition to the genetic variances and covariances of important traits [18, 22, 42]. Some kinds of nonadaptive environmental induction might affect the probability of ecological speciation. In particular, suboptimal development or noisy plasticity [45] in stressful environments could inhibit adaptation by decreasing the fitness of local relative to immigrant individuals. However, it would also increase the strength of selection and potentially result in cryptic adaptive divergence (countergradient variation) [18, 41].

Countergradient variation is negative covariance between genetic and environmental effects on phenotype [41]. Classic examples are poikilotherms such as fish and molluscs [89], flies [90], or frogs [91] that grow more slowly in cold climates, but cold-adapted populations have higher growth rates than warm-adapted populations when raised at the same temperature. The best explanation for this pattern is that genetic differences have evolved to compensate for divergent effects of environmental induction, resulting in populations that appear similar when measured each in their native habitat but show maladaptive plasticity when transplanted (Figure 3). Note that negative covariance between genetic and environmental effects is not necessarily maladaptive, but environmental effects will tend to be maladaptive if the optimum phenotype is roughly constant across the environment range.
When countergradient variation exists, we expect immigrants to have a fitness disadvantage owing to under- or overexpression of an environmentally sensitive trait relative to a local optimum (Figure 3). This might well promote evolution of restricted or nonrandom dispersal; hence intrinsic barriers to gene exchange as a result of divergent ecological selection. However, countergradient variation will evolve only if selection is stronger than gene flow and if reaction norms are genetically constrained (otherwise, we might expect adaptive evolution to flatten the reaction norm). At least in the early stages of colonization of a challenging new habitat, nonadaptive plasticity (such as stunted growth or suboptimal metabolic rates) might make resident individuals less viable and fecund than healthy immigrants from a less stressful habitat [22]. This potential fitness asymmetry could offset effects of adaptive genetic changes on the relative fitness of immigrants and residents. That is, offspring of immigrants might have lower fitness than offspring of native genotypes with locally adaptive alleles. However, offspring of immigrants might nevertheless outnumber offspring of natives if immigrants come into the stressful habitat with substantial viability and fertility advantages from being raised in a higher quality habitat. Parental care and physiological maternal effects could further extend those environmentally induced advantages to the offspring. The net effect could be a tendency for locally adapted genotypes to be replaced (“swamped”) by immigrants owing to a negative covariance between environmental and genetic effects. This effect of nonadaptive plasticity is synergistic with the potential for demographic swamping, a well-known constraint on local adaptation to novel habitats [74, 76, 87, 92].

For now, it appears that the impact of nonadaptive plasticity on the probability of ecological speciation cannot be predicted without additional detailed knowledge. Just as gene flow can constrain or facilitate local adaptation [74, 76], and adaptive plasticity can inhibit or promote adaptive genetic divergence [22, 27, 36], nonadaptive plasticity might impede genetic divergence by accentuating fitness advantages of immigrants and/or promote divergence by increasing the intensity of selection.

5. Phenotypic Plasticity and the Evolution of Reproductive Isolation

Plasticity of a different sort might directly affect reproductive compatibility between populations developing in different environments. Environmental induction might generate differences in preference, reproductive phenology, or expression of secondary sexual characteristics. Coincidence of environmentally induced reproductive barriers and potentially divergent selection can be genetically equivalent to a geographic barrier between divergent environments [59, 93, 94]. A key element of the developmental plasticity model of ecological speciation is the establishment of a consistent relationship between the environment of parents and that of their offspring. Similarity of parent and offspring environments maintains shared environmental effects on phenotype, consistency of selection, and reduces gene flow.

Environmental similarity can be a byproduct of geographic isolation or might be promoted by environmentally induced variation in habitat choice, phenology, or other aspects of mating behavior. Whether or not ecological speciation ensues depends on whether genetic reproductive barriers evolve and whether that evolution can be attributed to divergent selection.

A seemingly commonplace example of environmentally induced barriers to gene flow is flowering time in plants. Flowering time is often accelerated or delayed when a given genotype is grown on different substrates [34]. For example, grasses and monkey flowers colonizing contaminated soils around mines show environmentally induced shifts in flowering [95, 96], and palm trees on Lord Howe Island show soil-dependent flowering times [60]. These examples are also widely recognized cases of ecological speciation. Gavrilets and Vose [59] used simulations to explore the impact of environmentally induced shifts in flowering time on the probability of adaptive divergence and ecological speciation and confirmed that this instantaneous barrier to gene flow between habitats can markedly increase the probability and rate of divergence (Figure 4).

A similar effect arises owing to behavioural imprinting [97, 98]. Many animals, such as birds and anadromous fishes, are known for imprinting on their natal habitat [99–101]. When this is a direct matter of memorizing where home is (as might be the case in Ficedula flycatchers [101]), it simply accentuates the relationship between geography and gene flow. Slightly different implications emerge from imprinting on a kind of habitat, host, or resource because then the environmental influence on gene flow is independent of geography. Some phytophagous insects imprint on their

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**Figure 4:** Effects of phenotypic plasticity of flowering time on ecological speciation. The lines illustrate flowering periods for plants on two soil types (dashed and solid) based on initial conditions in the simulations of Gavrilets and Vose [59] for ecological speciation based on the **Howea** palm tree study [60]. I illustrate the case where flowering time is affected by 8 loci, and the environmental effect of soil type is either 0, 2, or 4 weeks difference. In the simulations [59], changing the number of loci has confounding effects on the initial environmental variance component and the fitness effects of mutations, so a single genetic scenario is illustrated here for simplicity. I calculated the initial rate of gene flow between soil types as \( m = 0.5 \) (% time overlap), based on the assumption that any tree is equally likely to receive pollen from any other currently flowering tree. %speciation is based on the number of simulations ending in speciation, given in Table 2 of Gavrilets and Vose [59].
host plant species based on chemical cues [102], and nest-parasitic indigobirds imprint on their hosts [103]. In these examples, phenotypic plasticity helps maintain similarity between maternal and offspring environments, which is important both for maintaining phenotypic similarity and consistency of selection on parents and offspring. However, it is not always obvious that habitat or resource imprinting should directly affect mating preferences. In the *Vidua* indigobirds, there is an imprinting effect of host song, such that nest parasites raised by the same host species tend to mate assortatively owing to learned elements of their own songs and preferences [103, 104]. In many phytophagous insects, mating occurs on or near the host plant [102, 105], making plant choice a “magic trait” simultaneously effecting ecological and sexual differentiation [31]. As with genetically determined traits, phenotypic plasticity of traits directly linked to both ecological adaptation and assortative mating is most likely to contribute to ecological speciation.

Environmental effects on traits directly involved in sexual selection are not unusual. Expression of pigments, pheromones, and other displays can depend on diet, condition, or experience [19, 106]. For example, premating isolation is induced by larval host plant differences in *Drosophila mojavensis*, because the chemical properties of their cuticular hydrocarbons (important contact pheromones) are strongly influenced by diet [107, 108]. Sharon et al. [109] recently showed that mate choice in *D. melanogaster* can be modified by symbiotic gut bacteria. Flies raised on a high-starch medium had microbiota dominated by *Lactobacillus plantarum*, which was only a minor constituent of the microbiota of flies raised on a standard cornmeal-molasses medium. Sharon et al. [109] found that the differences in bacterial composition can affect cuticular hydrocarbon levels, providing a probable mechanism affecting mate choice.

In Sockeye Salmon (*Oncorhynchus nerka*), postmating isolation might be caused by sexual selection on diet-derived coloration. Anadromous sockeye sequester carotenoids from crustaceans consumed in the ocean and use them to express their brilliant red mating colors. The nonanadromous morph (kokanee) expresses equally bright red mating color on a diet with much less carotenoids, an example of countergradient variation [110]. Anadromous morphs and hybrids raised in the freshwater habitat of the kokanee (low-carotenoid diet) underexpress the red pigment and probably suffer reduced mating success as a consequence [111, 112].

The actual effect of phenotypic plasticity on gene flow depends on environmental similarity between parents and offspring. Environmentally induced mate discrimination will have little or no hindrance on gene flow unless it also affects the phenotypes and/or environments of the offspring. For example, imagine a phytophagous insect with environmentally induced contact pheromones causing perfect assortative mating between individuals raised on the same host plant. If there are no differences in host choice, then the offspring of each mating type are equally likely to grow up on each plant and therefore have no tendency to develop the same phenomone profile as their parents (Figure 5). In this hypothetical case, there is free gene flow despite assortative mating of phenotypes.

Environmentally induced differences in habitat choice reduce gene flow when individuals are more likely to mate with other individuals using the same habitat, and offspring are more likely to grow up in habitats similar to those of their parents. If habitat choice is entirely determined by the individual’s environment (i.e., if there is no tendency for the offspring of immigrants to return to their parent’s original habitat), then the effect is genetically identical to a geographic barrier. Either way we can describe the system in terms of populations of individuals or gametes with some probability (m) of “moving” from their natal population to breed in a different population. In the simple case of two environments and nonoverlapping generations, the expected frequency of an allele in generation t in environment i is a weighted average of the frequencies in environments i and j in generation t − 1 [113]:

$$p_{ii(t)} = (1 - m)p_{ii(t-1)} + mp_{jj(t-1)}.$$  \((1)\)

It makes no difference whether m is determined by geography or environmental induction as long as there are not heritable differences in m among individuals within a habitat. More generally, geographic or spatial covariance is a special case of environmental similarity, and the extensive knowledge from decades of conceptual and mathematical modeling of gene flow’s effects on adaptation and speciation [30, 31, 87, 114, 115] can be extended directly to include this kind of phenotypic plasticity. In particular, we might expect environmentally induced restrictions on gene flow to facilitate the evolution of postzygotic incompatibilities (both environment–dependent and -independent selection against hybrids) and genetic assimilation of behavioral reproductive barriers (habitat and mate choice), but also to lessen the potential for selective reinforcement of assortative mating (just as adaptive plasticity lessens divergent selection on ecological phenotypes).

### 6. Conclusions and Future Directions

Opinions still seem to outnumber data about the impact of environmental induction and plasticity on evolution, but substantial progress has been made in the last 20 years [23, 64]. Plasticity appears to be a common if not universal feature of developmental systems and should not be ignored. Plasticity and environmental effects were once black boxes, ignored by some, uncritically promoted as threats to evolutionary theory by others. But theoretical and empirical investigations have increasingly shed light on how plasticity evolves and interacts with natural selection. Whether developmental plasticity rivals mutation as a source of quantitative or qualitative change (the evolution of “novelty”) remains contentious [17, 80, 116]. However, to the extent that speciation is defined by genetic divergence, genes will not be displaced from their central role in the study of speciation. Phenotypic plasticity can promote or constrain adaptive evolution and ecological speciation. The effects of plasticity in a particular case, and whether there is an overall trend, are empirical questions.

Important theoretical challenges for understanding the importance of plasticity for adaptation and speciation...
include the extension of models (such as [36]) to incorporate nonadaptive plasticity and countergradient variation, further investigation of how costs of plasticity affect genetic assimilation and reproductive isolation, and careful examination of what kinds of environmental effects on phenotype can be considered equivalent to geographic restrictions of gene flow (environmental effects on dispersal) [59, 93, 94, 114]. Not all environmental effects on mate choice will affect gene flow (Figure 5). However, maternal effects and factors that promote environmental similarity between relatives seem particularly likely to reduce gene flow and promote adaptive divergence. Incorporation of such effects into the classic models of local adaptation [87] and ecological speciation [31] might bring substantial clarification to the subject. Moreover, how environmentally induced barriers to gene flow might affect the evolution of constitutive genetic barriers has not been explored. Should we expect environmental effects on assortative mating to slow the evolution of genetic barriers to gene flow? Finally, virtually no attention has been given to the relationship between plasticity and postzygotic isolation. Key questions include how might plasticity affect ecologically based selection on hybrids, and are highly plastic developmental pathways good or bad candidates for involvement in postzygotic developmental incompatibilities?

Addressing empirical challenges might best begin by establishing criteria for recognizing plasticity as a causal factor in speciation or adaptive radiation. West-Eberhard [15] and Pfennig and McGee [117] suggested that associations between intraspecific plasticity and species diversity support a role for plasticity in promoting adaptive radiation. Examples like the tiger salamander radiation (Figure 1) and others [117], where an intraspecific polyphenism parallels a repeated pattern of interspecific divergence, are consistent with adaptive plasticity as an origin of ecological divergence between species. More confirmatory evidence for a causal role of plasticity in adaptive divergence (the first component of ecological speciation) would come from testing the strengths of tradeoffs, costs of plasticity, and selection against

Figure 5: Free gene flow despite assortative mating. If mating cues are environmentally induced but there is no habitat choice, host-associated populations will not be genetically differentiated, and it takes two generations to establish Hardy-Weinberg equilibrium. If the host densities are \( p \) and \( 1 - p = q \), a locus that is fixed for different alleles on different hosts has allele frequencies \( p \) and \( q \). At first, genotypes are perfectly associated with the host-induced mating cue, but random dispersal eliminates that association in one generation if there are no other factors maintaining covariance between parent and offspring phenotype (e.g., maternal effects or divergent selection). Once genotype frequencies are equalized between phenotypes, mating within phenotypes establishes Hardy-Weinberg genotype proportions [61]. Although imagining a locus with complete differentiation makes for the simplest illustration, the result is completely general for any allele frequency. If we somehow know what alleles have ancestors in each habitat in generation 0, the result is that it takes just two generations to completely randomize that ancestry, regardless of whether or not the alleles are actually different by state.

A. 1st generation: Larvae on different host plants develop environmentally induced mating cues represented by red and blue.

B. Random dispersal results in equal frequencies on each host plant. Association between genotype and mating phenotype persists.

C. 2nd generation: Larvae produced by assortative mating deviate from Hardy-Weinberg, but develop environmentally induced mating cues.

D. Random dispersal maintains equal frequencies. There is no association between genotype and mating phenotype

E. 3rd generation: Hardy-Weinberg genotype proportions are expected for larvae on each host despite assortative mating by environmentally induced phenotype.
immigrants and hybrids. Effects of plasticity on reproductive isolation (the second component of ecological speciation) are illustrated in studies like those of parasitic indigobirds [103, 104] and *Drosophila* [107–109] on environmentally induced barriers to gene flow. We presently know little of the prevalence of this phenomenon or whether it is often strong enough to facilitate substantial genetic differentiation. Field studies are needed to document the prevalence and strength, in nature, of costs of plasticity, evolvability of reaction norms, and environmental effects on reproductive isolation. The principle illustrated in Figure 5 should be considered in the design and interpretation of experiments. In order for plasticity of mating behaviour to directly impact gene flow, there must be some factor maintaining similarity among immigrant and hybrids. E
c

The theoretical and empirical foundations of speciation research are very strong in terms of genetics and geography [29–31, 115]. The roles of ecology, environment, and development are prominent among the remaining frontiers [27, 36, 52]. Further research integrating genetics, ecology, and development promises great gains in understanding the origins of biological diversity.

**Acknowledgments**

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**References**


[107] M. D. Sten nett and W. J. Etges, “Intraspecific divergence is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. III. Epicuticular hydrocarbon variation is determined by use of different host plants in *Drosophila mojavensis*.


Review Article

Ecological Adaptation and Speciation: The Evolutionary Significance of Habitat Avoidance as a Postzygotic Reproductive Barrier to Gene Flow

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Habitat choice is an important component of most models of ecologically based speciation, especially when population divergence occurs in the face of gene flow. We examine how organisms choose habitats and ask whether avoidance behavior plays an important role in habitat choice, focusing on host-specific phytophagous insects as model systems. We contend that when a component of habitat choice involves avoidance, there can be repercussions that can have consequences for enhancing the potential for specialization and postzygotic reproductive isolation and, hence, for ecological speciation. We discuss theoretical and empirical reasons for why avoidance behavior has not been fully recognized as a key element in habitat choice and ecological speciation. We present current evidence for habitat avoidance, emphasizing phytophagous insects, and new results for parasitoid wasps consistent with the avoidance hypothesis. We conclude by discussing avenues for further study, including other potential roles for avoidance behavior in speciation related to sexual selection and reinforcement.

1. Introduction

The last two decades have seen a renewal of interest in the ecological context of speciation [1–5]. This interest has been spawned, in large part, by a greater acceptance that speciation can progress in the face of gene flow [6–10]. Ecological adaptation is a central component of many speciation-with-gene-flow models [8]. Specifically, divergent natural selection imposed by different habitats or environments can serve as an important “extrinsic” barrier to gene flow to initiate speciation [11]. When fitness tradeoffs exist, populations may not always be capable of simultaneously adapting phenotypically and genetically to the contrasting selection pressures imposed by different habitats to be jack-off-all-trades generalists. Consequently, migrants moving between habitats or hybrids formed by mating between individuals from different populations will not thrive compared to resident parental types [12]. Thus, ecological reproductive barriers due to fitness trade-offs can form that, if strong enough, can promote speciation.

Two aspects of ecological adaptation are particularly important for speciation-with-gene-flow. One concerns habitat-related performance traits and the other habitat choice [13–17]. Habitat performance involves traits or genes that increase the survivorship of their progeny in one habitat but have negative fitness consequences in alternative habitats. Such tradeoffs have obvious consequences for reducing effective gene flow between populations due to the reduced fitness of migrants and hybrids, as discussed previously. Questions have been raised as to how common and strong performance tradeoffs are between populations in nature [18–20]. However, instances of strong host plant-related fitness tradeoffs for plant-eating insects have been found [8, 21]. Moreover, it is clear that certain ecological dimensions of habitats will likely impose strong divergent selection pressures. For example, morphological aspects of resource
processing such as the beaks of birds [22–24], the proboscis length of insects [25], and the pharyngeal gills of fish [26] will not be ideal for all food items and will incur a cost in handling time to effectively use a subset of resources versus others. Also, seasonal differences in resource availability cannot always be bridged by a single life history strategy for a short-lived organism that, for instance, has only a single generation per year [27]. In addition, aspects of pollinator attraction [28] and crypsis in relation to the environment [29] can also have consequences for fitness. Thus, there are reasons to suspect that several ecological dimensions of habitats can impose strong and divergent selection pressures on populations to most effectively utilize them.

The second component of ecological adaptation concerns habitat choice. When habitat-related performance differences exist, individuals that prefer to reside in the habitat to which they are best adapted benefit from a fitness advantage [15]. When habitat choice affects mate choice, then the result is an ecologically based barrier to gene flow. This most easily occurs when individuals mate in their preferred habitats (e.g., if populations form leks or court in different habitats or at different times of the year). The resulting assortative mating can facilitate ecological speciation-with-gene-flow because it accentuates selection for performance traits that in turn increase selection for habitat fidelity [15–17]. By mating in preferred habitats, individuals that possess traits increasing their survivorship in a particular habitat will tend to mate with other individuals possessing the same suite of ecological adaptations, increasingly favoring those that remain in their “natal” habitats. Thus, positive correlations can evolve between traits affecting habitat choice (e.g., host plant quality decision for oviposition by phytophagous insects) and offspring survival in habitats [30].

Here, we are interested in how organisms choose habitats. Specifically, we ask the question of whether avoidance behavior plays an important role in habitat choice, focusing on host-specific phytophagous insects as model systems. We contend that when a component of habitat choice involves avoidance, there can be repercussions that can have consequences for enhancing the potential for specialization and postzygotic reproductive isolation and, hence, for ecological speciation.

2. Habitat Preference versus Avoidance

In general, when we think about how organisms decide to reside in one habitat versus alternatives, we think in terms of positive preference. Specifically, preferences exist that result in an individual deciding to live in one habitat that it likes the best over others. If we think about preferences being affected by some number of genetic loci, then a greater proportion of alleles across these preference genes favoring the acceptance of one habitat versus another would result in an organism being relatively more likely to reside in that habitat [16]. Hybridization between populations with preferences for different habitats will result in offspring of mixed ancestry that will, in the absence of genetic dominance, tend to have no strong preference for either parental habitat and consequently an interest for both habitats. These hybrids could therefore serve as effective bridges or conduits of gene flow between populations in different habitats and impede specialization. For this reason, Mayr [31, 32] argued that the genetics of habitat preference was a problem for ecological speciation-with-gene-flow. The more preference genes that affect habitat choice in the genome, the harder it would be for pure habitat preference for one parental habitat versus another to sort itself out in individuals of mixed ancestry. Thus, it would be difficult for ecological specialization to drive speciation in the face of gene flow unless habitat choice was concentrated in just a few loci of strong effect with consistent dominance interactions for choice of a particular habitat.

Habitat choice may not be solely determined by positive preference, however. It has long been argued that phytophagous insect specialists, for example, use positive cues from hosts and negative cues from nonhost plants when evaluating their environment [33–35]. Consequently, as well as possessing genes to prefer specific habitats, phytophagous insects may also have loci or alleles at certain loci that cause them to avoid nonnatal, alternate habitats.

Theoretical models have been constructed that have considered habitat avoidance as a factor in ecological speciation but these have been restrictive in that they tend to model avoidance additively [36, 37]. However, avoidance (as well as preference) may commonly have nonadditive phenotypic and fitness effects with important consequences for ecological speciation that go beyond the implications of habitat preference. For example, alleles resulting in increased preference for one habitat may have pleiotropic consequences simultaneously increasing an individual’s disdain for other nonnatal habitats that do not possess preferred characteristics [17]. Also, if avoidance genes exist, then hybrids possessing alleles to avoid alternate parental habitats may be behaviorally conflicted and accept no habitat. Thus, hybrids could suffer a degree of postzygotic reproductive isolation, being incapable of finding suitable habitats to feed and mate in (i.e., hybrids would incur a degree of behavioral inviability/sterility). Moreover, in contrast to preference genes, the more genes there are that strongly and independently affect avoidance, the stronger the barrier to gene flow between populations, as it would become harder and harder to segregate out a parental behavioral phenotype that would be willing to reside in any one habitat [17]. Thus, rather than the genetics of habitat choice providing a bridge, avoidance could create a greater reproductive chasm fostering ecological speciation-with-gene-flow.

Avoidance could also have consequences for ecological speciation not only occurring in the face of gene flow in sympatry or parapatry but also occurring in allopatry in geographically isolated populations. If the evolution of increased preference has pleiotropic effects that increase avoidance for alternative habitats, then the independent evolution of increased natal habitat choice in allopatry might cause both pre- and postzygotic isolation if populations were ever to come back together in secondary contact. In this case, the evolution of increased preference in allopatry could stem, for example, from selective pressures for individuals to more efficiently and accurately judge variation in their
natal habitat quality and avoid suboptimal natal resources [38–40]. Alternatively, avoidance could also initially evolve in response to local nonnatal habitats that become significant following secondary contact when these alternate habitats share characteristics in common with other, previously allopatric, nonnative habitats inhabited by a related taxon.

The issue of why habitat avoidance is not a more generally appreciated element of ecological adaptation is a central topic of this paper. We argue that part of the answer may be theoretical and part due to experimental design. For avoidance to cause postzygotic reproductive isolation in sympatry or parapatry invokes the classic theoretical problem that a new mutation causing inviability or sterility cannot establish in the face of gene flow [41]; any new mutation reducing fitness in heterozygotes, including behavioral avoidance incompatibilities in hybrids, should be rapidly selected against and eliminated from populations. In this paper, we propose several ways in which this problem can be alleviated and that avoidance behavior can evolve in the face of gene flow despite negative fitness consequences in hybrids. This includes a discussion on the potential genetic and physiological changes that can result in the evolution of habitat avoidance, focusing on the olfactory system of host plant specific phytophagous insects.

The second problem with avoidance is empirical. Although the hypothesis of habitat avoidance has been articulated, in part, by several leading entomologists including Bernays and Chapman [33], Jermy and Szentesi [42], and Frey and Bush [43], the idea has overall gained only modest experimental traction (see [34]). We suggest that this may stem from studies of habitat use generally not being designed or analyzed with the possibility of avoidance behavior in mind. Rather, most host choice experiments are focused on preference and whether hybrid individuals will accept one parental habitat (or host plant) versus the other. Evidence for avoidance can go undetected or unexamined in such tests. Here, we discuss several studies of insect host plant choice in the literature and new results from a wasp parasitoid *U. lectoides* that are consistent with the evolution of host avoidance. We conclude by examining several aspects of host plant choice studies that may mask the presence of avoidance behavior and suggest that avoidance may be a common component of ecological specialization and speciation, at least for phytophagous insect specialists.

3. Theoretical Models for Habitat Avoidance

The basic theoretical issue with habitat avoidance generating behavioral inviability/sterility concerns the following conundrum: how is it possible, if hybrids are unfit, to evolve from the high-fitness genotype of one specialist population (species) to the high-fitness genotype of the other population (species) without passing through a low fitness intermediate? In allopatry, the answer is relatively straightforward and is attributed to the insights of Bateson, Dobzhansky, and Muller [44–47]. Specifically, hybrid inviability or sterility often arises from between-locus genetic incompatibilities: alleles that function well within species are incompatible with one another when brought together for the first time in the genome of an individual of mixed ancestry ([48] for review). Genetic architectures of this form are known as Dobzhansky-Muller (D-M) incompatibilities and allow populations to evolve reproductive isolation without passage through a fitness-valley that would be opposed by selection. Alleles for preference for the natal habitat that inadvertently cause avoidance to alternative, nonnatal habitats accumulate independently in allopatric populations separated by geographic barriers that preclude migration and, consequently, no negative fitness consequences in hybrids. If these physical barriers happened to dissipate and populations were to come back into secondary contact and interbreed, then hybrids would suffer behavioral inviability/sterility due to possessing alleles causing them to avoid aspects of all suitable parental habitats. Thus, avoidance can easily be envisioned to play an important role in generating ecological reproductive isolation in allopatry.

We note that in allopatry, a similar argument can also be made with respect to mate choice. Sexual selection and assortative mating might involve both a preference for a given trait(s) in mates and avoidance of individuals lacking the trait(s). Hence, avoidance aspects of mate choice can result in both pre- and postzygotic reproductive isolation following secondary contact in a similar manner as ecological habitat choice. Moreover, the same considerations with respect to the number of loci underlying habitat avoidance would apply to mate avoidance; the greater the number of genes that independently affect avoidance, the potentially stronger the reproductive barrier to gene flow. The role of avoidance behavior in sexual selection and assortative mating is an area warranting further theoretical study.

The theoretical difficulty with habitat avoidance therefore rests primarily on whether similar behavioral inviability/sterility barriers can also evolve in sympatry or parapatry in the face of gene flow, as is possible in the absence of migration in allopatry. Here, the argument is that it is not feasible because unlike the situation in allopatry, the negative consequences of a new mutation causing avoidance would be immediately exposed to selection against it in heterozygotes. However, there are reasons why this conventional wisdom may be incorrect. First, parapatric models have shown how new favored mutations that can cause genomic incompatibilities can alternately arise in the nonoverlapping portions of two taxon’s ranges experiencing low levels of migration and elevate to high frequencies (e.g., see [49]). When these favored mutations spread into the contact area, hybrids suffer reduced fitness and thus a reproductive barrier to gene flow exists that fosters speciation. Consequently, with certain parapatric distributions of taxa, behavioral habitat inviability/sterility can evolve in a similar manner as in allopatry. But in these circumstances, avoidance behavior is not initially evolving directly in the face of gene flow.

Second, Agrawal et al. [50] in this issue show that when new mutations subject to divergent natural selection also affect intrinsic isolation, either directly or via linkage disequilibrium with other loci, such alleles can overcome gene flow, diverge between populations, and thus result in the evolution of intrinsic isolation. Thus, if divergent
selection for nonnatal habitat avoidance is strong enough relative to migration and to its deleterious consequences in heterozygote “hybrids,” it can evolve in the face of gene flow to differentiate sympatric and parapatric populations. Increased habitat fidelity could be favored to restrict the formation of less fit hybrids, even if it involved a degree of increased behavioral inviability/sterility in individuals of mixed ancestry, provided that the increase in fitness due to pre-zygotic isolation outweighs the postzygotic cost. Indeed, a difficulty with reinforcement models is that with decreased hybridization due to pre-zygotic isolation, the selection pressure for the evolution of further reinforcement decreases [51]. However, if the differential establishment of avoidance alleles in divergent populations increases not only pre-zygotic isolation but also the degree of behavioral inviability/sterility in hybrids, then selection pressures for reinforcement could remain strong even as gene flow levels decrease. The role of avoidance behavior in reinforcement is an area in need of further theoretical analysis.

Third, the divergent selection models of Agrawal et al. [50] in this issue mainly focus on whether a new favorable mutation conferring a fitness advantage in one habitat could establish in the face of gene flow if it directly interacted negatively with other alleles in the prestanding genetic background to cause postzygotic behavioral inviability/sterility. However, much like traditional D-M incompatibilities [41], the evolution of avoidance behavior could involve a stepwise sequence of substitutions of new mutations that each resulted in a minimum amount of reduced fitness in heterozygotes or hybrids until completion of the process. As a consequence, sequential habitat choice models might widen the conditions for divergent ecological selection to generate postzygotic reproductive. We depict three such sequential habitat choice models in Table 1 that involve either intermediate alleles and dominance interactions at a single locus or epistatic interactions between loci to cross through fitness valleys for habitat avoidance to evolve and generate postzygotic isolation.

The first model (see Figure 1 and Table 1) involves multiple substitutions at a single locus affecting habitat choice in which one of the allelic states (o) at the choice gene is a null mutation that does not affect habitat choice. This model is an

<table>
<thead>
<tr>
<th>Table 1: Three models for how genetically based behavioral incompatibilities due to conflicting habitat avoidance can evolve during speciation-with-gene-flow that cause postzygotic isolation in hybrids (see Figures 1–3).</th>
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<tbody>
<tr>
<td><strong>Model 1: intermediate null allele (o)</strong></td>
</tr>
<tr>
<td>Step 1: a’ state in ancestral population for a habitat choice locus with a recessive null (o) allele also segregating as low a frequency polymorphism that confers no habitat preference.</td>
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<tr>
<td>Step 2: new habitat becomes available and o/o genotypes from ancestral population shift and adapt to novel habitat.</td>
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<tr>
<td>Step 3: new avoidance mutation to ancestral habitat (a’) arises and elevates to high frequency in primarily o null allele initial genetic background of novel habitat population.</td>
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<tr>
<td>Step 4: new avoidance mutation to novel habitat (n’) arises that confers even greater fidelity to ancestral habitat than a’ allele and elevates to high frequency in the ancestral habitat population.</td>
</tr>
<tr>
<td>Step 5: behavioral inviability/sterility in hybrids when individuals move between novel and ancestral habitat and cross mate producing n’/a’ heterozygotes at habitat choice locus.</td>
</tr>
<tr>
<td><strong>Model 2: epistasis between habitat preference and avoidance loci</strong></td>
</tr>
<tr>
<td>Step 1: initial a’ state exists for ancestral habitat preference at ancestral preference locus in ancestral population and initial n’ state for novel habitat avoidance at habitat avoidance locus.</td>
</tr>
<tr>
<td>Step 2: new habitat becomes available and new preference mutation for novel habitat (n’) arises and elevates to high frequency in novel habitat population.</td>
</tr>
<tr>
<td>Step 3: new avoidance mutation to ancestral habitat (a’) arises at avoidance locus and elevates to high frequency in novel habitat population because negative effects of n’/a’ heterozygosity at avoidance locus are not expressed in n’/n’ genetic background at the preference locus in the novel habitat.</td>
</tr>
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<td>Step 4: behavioral inviability/sterility in hybrids when individuals move between novel and ancestral habitat and cross mate producing n’/a’ heterozygotes at habitat avoidance locus in n’/a’ genetic background of hybrids at the preferrelocus.</td>
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<tr>
<td><strong>Model 3: epistasis between ancestral and novel habitat loci with preference and avoidance alleles</strong></td>
</tr>
<tr>
<td>Step 1: initial a’ state exists for ancestral habitat preference at ancestral habitat choice locus in ancestral population and initial n’ state for novel habitat avoidance at habitat avoidance locus.</td>
</tr>
<tr>
<td>Step 2: new habitat becomes available and new preference mutation for novel habitat (n’) arises at novel habitat choice locus and elevates to high frequency in novel habitat population.</td>
</tr>
<tr>
<td>Step 3: new avoidance mutation to ancestral habitat (a’) arises at ancestral habitat choice locus and elevates to high frequency in n’/n’ genetic background at the novel habitat locus in the novel habitat population.</td>
</tr>
<tr>
<td>Step 4: behavioral inviability/sterility in hybrids when individuals move between novel and ancestral habitat and cross mate producing a’/a’ heterozygotes at ancestral habitat locus in n’/a’ genetic background of hybrids at the novel habitat locus.</td>
</tr>
</tbody>
</table>
Figure 1: Illustration of model 1 based on intermediate null allele (see Table 1 for model description). Numbered steps of model (1–5) are separated by dashed lines and ordered vertically. Individuals are represented by chromosome pairs as grey bars; avoidance locus is marked by solid black tick mark on grey chromosome. Alleles at avoidance locus are labeled as follows: $a^+$: preference for ancestral habitat; $o$: recessive null allele (i.e., no preference or avoidance); $a^-$: avoidance for ancestral habitat; $n^-$: avoidance for novel habitat. Arrow depicts shift from ancestral habitat 1 to novel habitat 2.
extension of Nylin and Wahlberg’s [52] hypothesis that host shifts for phytophagous insects start with a broadening of the host acceptance range (e.g., ovipositing females respond to a wider range of plant cues). Following this initial generalist (oligophagous) phase, sympatric speciation can occur via host race formation or allopatric speciation can occur via the evolution of more specialized local subpopulations [53, 54]. For example, initially most individuals attacking an ancestral host plant may possess $a^+$ alleles conferring ancestral host recognition. However, a recessive null ($a$) allele resulting in no habitat preference may also initially be present in the ancestral population as standing genetic variation (see Step 1 for Model 1 in Figure 1 and Table 1). In the absence of an alternative host, the null allele might have little negative fitness consequences to its bearers and so exists as a low-frequency polymorphism maintained by purifying selection/mutation balance. An alternative, novel habitat then becomes available and is colonized primarily by individuals with the recessive ($a$) allele (Step 2). After some local (performance-related) adaptation to the novel habitat, a new dominant avoidance allele ($a^-$) can arise and reach high frequency in the novel population to avoid the ancestral habitat where migrants would have lower fitness (Step 3). Following this, if a new mutation for strongly avoiding the novel habitat ($n^-$) happened to arise in the ancestral population, then it could reach high frequency in the ancestral population (Step 4). Rather than being primarily selected against in an $a^-$ genetic background in which the negative fitness consequences of conflicted host choice would be immediately exposed in heterozygotes, the $n^-$ allele would mainly be selected for in the ancestral population in an $a^+$ genetic background. Thus, alternative $a^-$ and $n^-$ alleles could come to differentiate populations utilizing novel versus ancestral habitats that adversely interact with each other to decrease hybrid fitness (Step 5).

A second model (see Figure 2 and Table 1) involves two separate loci, one affecting habitat preference and the other habitat avoidance, that epistatically interact. In most genetic backgrounds, heterozygotes for alternate habitat avoidance alleles to the novel and ancestral habitats ($n^-/a^-$) at the habitat avoidance locus would have impaired behavior. However, in an $n^+/n^+$ homozygous genetic background for the preference locus causing individuals to orient to the novel habitat (Step 2), the deleterious phenotypic effects of $n^-/a^-$ heterozygosity at the avoidance locus are not expressed (Step 3), providing a pathway to alleviate the maladaptive fitness valley associated with habitat avoidance during a shift to a novel habitat (Step 4).

Finally, a third model (see Figure 3 and Table 1) involves epistatic interactions as in model two, except rather than separate preference and avoidance loci, habitat choice is based on one locus that influences orientation to the ancestral habitat and a second that dictates choice of the novel habitat. The distinction between models 2 and 3 then is that in model 2 a given locus affects either avoidance or preference, but not both behaviors, to alternative habitats. In contrast, in model 3 the different loci are directly tied to cues associated with one habitat or the other (be they olfactory, visual, tactile, or gustatory), with different alleles conferring different avoidance or preference responses to just those habitat specific cues. In the case of model 3, an initial mutation at the novel habitat locus from $n^-$ to $n^+$ can result in a degree of orientation to the novel host (Step 2). In an $n^+/n^+$ genetic background, a new $a^-$ avoidance mutation to the ancestral host occurs at the ancestral habitat locus which establishes in the novel habitat (Step 3) and is only behaviorally incompatible in hybrids possessing $a^+$ and $n^-$ alleles at the ancestral and novel habitat choice genes, respectively (Step 4).

Feder and Forbes [17] theoretically analyzed the second epistasis model through a series of computer simulations in which levels of gene flow, divergent selection, habitat choice, recombination, and hybrid behavioral incompatibility were varied. The results implied that the conditions for habitat avoidance were not overly restrictive under the epistasis scenario and that the evolution of behavioral inviability/sterility in the face of gene flow was possible. More detailed analyses estimating the probabilities of establishment of new avoidance alleles are still needed to definitively assess how likely epistasis model two is for generating behavioral inviability/sterility with gene flow. The computer simulations were based on deterministic models showing that the frequencies of new avoidance alleles would increase due to selection but did not assess how probable this increase would be relative to stochastic loss. Moreover, analyses of the first and third models are still required, although preliminary deterministic simulations suggest that they have similar characteristics as model two (Feder, unpublished data). Consequently, while the evolution of genomic incompatibilities is undoubtedly easier in allopatry than in sympatry or parapatry, there is no strong theoretical impediment to habitat avoidance evolving and causing a degree of hybrid behavioral inviability/sterility.

4. Physiological Basis for Habitat Avoidance

A key question with respect to the issue of habitat avoidance is how organisms that are specialized for an ancestral habitat evolve the physiological mechanisms to not only prefer a novel habitat but also avoid their original ancestral habitat. In this regard, it would seem most likely that such a transition, if it evolved in the face of gene flow, would initially involve a large and rapid shift in behavior due to a small number of important alleles or loci, rather than a number of small, quantitative, stepwise changes. If habitat choice involved a large number of small effect genes, then this would imply that a relatively high degree of migration would be occurring between populations during critical early periods of speciation-with-gene-flow. In this case, unless all new mutations for choosing (or avoiding) the novel habitat were dominant and/or habitat-related performance differences were pronounced and genomically wide-spread, it would seem difficult for a number of small effect genes to establish. Moreover, it would make it theoretically more difficult for behavioral inviability/sterility to evolve, as the small advantage in habitat choice conferred by these genes could not be offset by any appreciable negative consequences in hybrids or the new mutations would not establish. Thus,
Figure 2: Illustration of model 2 based on epistasis between habitat preference and avoidance loci. Numbered steps of model (1–4) are separated by dashed lines and ordered vertically. Individuals are represented by chromosome pairs as grey bars; preference locus (on left) and avoidance locus (on right) are marked by two pairs of vertical black tick marks on grey chromosomes. Alleles at preference locus are labeled as follows: $a^+$: preference for ancestral habitat; $n^+$: preference for novel habitat. Alleles at avoidance locus are labeled as follows: $n^-$: avoidance for novel habitat; $a^-$: avoidance for ancestral habitat. Arrow depicts shift from ancestral habitat 1 to novel habitat 2.
Ancestral habitat locus

Novel habitat locus

F1 hybrid rejects habitat 1 due to $a^+/a^-$ effect unmasked in $n^+$/n$^-$ background

F1 hybrid rejects habitat 2 due to $a^+/a^-$ effect unmasked in $n^+$/n$^-$ background

Figure 3: Illustration of model 3 based on epistasis between ancestral and novel habitat loci with preference and avoidance alleles. Numbered steps of model (1–4) are separated by dashed lines and ordered vertically. Individuals are represented by chromosome pairs as grey bars; ancestral (on left) and novel (on right) habitat loci are marked by two pairs of vertical black tick marks on grey chromosomes. Alleles at ancestral locus are labeled as follows: $a^+$: preference for ancestral habitat; $a^-$: avoidance for ancestral habitat. Alleles at novel locus are labeled as follows: $n^+$: preference for novel habitat; $n^-$: avoidance for novel habitat. Arrow depicts shift from ancestral habitat 1 to novel habitat 2.
it would seem that if speciation with-gene-flow is to occur, initial changes in host choice may often involve large effect alleles. Of course, this is not an important consideration in allopatry, where habitat choice genes of small effect could accumulate independently in geographically isolated demes and have large deleterious behavioral consequences in hybrids following secondary contact and gene flow. Regardless, the issue remains of how a new mutation could induce a big (or even small) shift in behavior from a like to dislike (or vice versa).

We illustrate possible mechanisms for generating large behavioral shifts in habitat choice using the olfactory system of phytophagous insects as a model (see Figure 1 in supplementary material available online at doi: 10.1155/2012/456374.). We focus on olfaction in insects because it is well-established that many phytophagous insect specialists are highly sensitive to particular compounds [55–57] or characteristic mixtures of volatiles [35, 58, 59] emitted from their host plants. This olfactory sensitivity is normally viewed as an adaptation for host finding, especially when the herbivore displays directed, odor-based anemotaxis over the fruit of its host plant, *Morinda citrifolia* [33, 34, 42]. This suggests a possible role for olfactory avoidance in host plant choice and the potential for behavioral incompatibilities in hybrids. Moreover, there are more plant-associated insects (>$500,000 described species) than any other form of Metazoan life, making the issue of host avoidance particularly relevant for understanding the relationship between ecological adaptation and the genesis of biodiversity [60].

The peripheral olfactory system of insects consists of chemosensory neurons present in specialized sensory hairs called sensilla (Supplementary Figure 1) ([71] for review). In many insects, olfactory sensilla are found on two pairs of olfactory organs on the head, the antennae and the maxillary palps. Each olfactory sensillum is innervated by up to four olfactory receptor neurons (ORNs). Odor molecules from the environment pass through pores in the cuticular walls of the sensilla where they become bound to odor binding proteins (OBPs). The OBPs transport the odor molecules to seven-transmembrane bound odorant receptor (OR) proteins that span the cell membranes of the dendrites of ORNs. Individual ORNs express only one or a few ORs of a potential large superfamily of OR genes [72–75]. *Drosophila*, for example, possess 60 different OR genes in their genomes [76]. Each OR binds to a unique class of molecule or compound, which confers specific odor response properties to the firing of the ORN [73–75, 77]. Output from the peripheral olfactory system ORNs is sent to two antennal lobes that contain a number of nerve cells organized into glomeruli. In most cases, the innervated axons of ORNs expressing the same OR converge to a single glomerulus in each antennal lobe [72, 78]. Thus, the number of glomeruli is approximately equal to the number of OR genes an insect possesses. Here, the ORN axons synapse with second-order neurons that project to the higher brain centers in insects: the mushroom body and the lateral horn [79]. The glomeruli are also the locations of local interneuron synapses, which enable the flow of information between glomeruli and likely play roles in organizing the input signal [79, 80]. It is generally thought that the negative and positive neural inputs are processed in an additive manner in the central nervous system of insects [81], resulting in an insect behaviorally responding to whatever the balance of olfactory input signals is at a particular moment in time [35].

| Table 2: Changes to olfactory system that can potentially affect the behavioral orientation of insects to plants. |
|---|---|
| Genetic change | Effect |
| Odorant binding-protein conformation | Determines nonpolar compounds carried to dendrite of chemoreceptor |
| Change in conformation of protein at receptor site on chemoreceptor | Influences sensitivity of cell to particular compound |
| Alter numbers of receptor sites for particular chemical on chemoreceptor | Alter sensitivity of cell to particular compound |
| Alter second messenger or membrane properties of + or − chemoreceptor | Alter sensitivity of + or − cell overall |
| Alter gene expression pattern of receptor proteins on + and − cells | Switch effect of stimulation from + to − or vice versa |
| Alter wiring of sense cells or interneurons | Switch effect of stimulation from + to − or vice versa |
| Alter levels of one or more neuromodulators at synapses | Alter weighting of different + and − inputs |
| Alter sign of synaptic inputs in path to controlling center | Alter weighting of different + and − inputs |

Switches from a positive to negative response to a chemical input signal could conceivably occur due to changes at any one of several points in the olfactory system. (Note that there is a general similarity and deep evolutionary homology in the sensory systems between insects and other multicellular organisms, implying that changes in the insect can have parallels in many other life forms.) We outline a few potential target points in Table 2. In essence, the central component of the argument is that if areas of the insect brain are associated with positive orientation when innervated and others with avoidance, then a shift in behavior can occur through any mechanism that changes (switches) the input signals to these areas. Thus, a change in the expression pattern of OBPs or ORs genes in ORNs can result in an odorant that was formally an attractant (agonist) of behavior becoming a deterrent (antagonist) or vice versa. For example, oviposition site preference is determined by a few loci in the fruit fly, *Drosophila sechellia*, where an odorant-binding protein gene is involved in the specialization of the fly to the fruit of its host plant, *Morinda citrifolia* [85]. Similarly,
a developmental change in the connections of ORN axons to glomeruli or of the secondary order neurons to the higher brain centers would also produce such a transformation. Thus, there are several potential mechanisms in which a new mutation could have a major effect on chemotaxis.

An outstanding issue is still why a phytophagous insect’s olfactory system should not be more sophisticated and allow for fine-tuned and subtle host discrimination rather than the general black and white patterns of likes and dislikes that we cite previously. One answer, if our hypothesis is correct, may be that the level of complex decision making in olfactory (and sense) systems is context dependent on the neural capacity of organisms. Bernays [35] has argued that neurons are energetically expensive to operate and develop, as well as to house when body size is an issue. Thus, there may be constraints on how many nerve cells and how large and interconnected many phytophagous insect’s brain can be. As a result, many phytophagous insects may be limited to more basic agonistic (likes) versus antagonistic (dislikes) behavioral responses to host plant cues. Such a system can also make sense in how most phytophagous insects probably experience the world. It may not usually be the case that an insect will have to make a choice between two or more different potential host plants simultaneously. Rather, it may be more common in typical spatially heterogeneous environments for an individual to experience a single host at a time and have to decide whether to accept or reject it. Thus, although we do not expect that all insect olfactory responses are black/white in nature, such decision-making system, while perhaps not always optimal, may be a serviceable solution for host choice for many insects given the inherent evolutionary constraints.

5. Empirical Studies

Our motivation for considering the role of habitat avoidance in ecological speciation is due, in part, to empirical discoveries in the apple maggot fly, *Rhagoletis pomonella*, a model system for sympatric speciation via host shifting [1, 86–88]. In particular, the recent shift of the fly from its native and ancestral host hawthorn (*Crataegus* spp.) to introduced, domesticated apple around 150 years ago in the eastern U.S. is often cited as an example of incipient sympatric speciation in action [8].

Volatile compounds emitted from the surface of ripening fruit have been shown to be the most important long to intermediate range cues used by *R. pomonella* to locate host trees [89]. Once in the tree canopy, flies use both visual and olfactory cues at distances of ≤1 meter to pinpoint the location of fruit for mating and oviposition. Recently, we found that not only are the ancestral hawthorn and recently formed apple-infesting host races of *R. pomonella* attracted to volatile compounds emitted from their respective natal-host fruit [90], but they also tend to avoid the nonnata! volatiles of the alternate fruit [64, 65]. Because *R. pomonella* flies mate only on or near the fruit of their respective host plants [91, 92], fruit odor discrimination results directly in differential mate choice.

Most interestingly, F1 hybrids between apple and hawthorn flies failed to orient to the odor of either apple or hawthorn fruit in flight tunnel assays, consistent with behavioral conflicts generated by fruit volatile avoidance [82]. In addition, behavioral responses to parental fruit volatiles were restored in a fair proportion of F2 and backcross hybrids (35–60%), suggesting that only a few major genetic differences underlie the phenotype [93]. This does not mean that a large number of genes do not affect olfaction and host choice. Rather, it suggests that only that a few changes in a relatively small number of genes might generate behavioral incompatibilities. Field tests still need to be conducted to determine if the compromised chemosensory system of nonresponding hybrids results in greatly reduced fitness in nature, although it is difficult to envision how these flies do not suffer at least some disadvantage. We also caution that the *Rhagoletis* results do not directly confirm that conflicting avoidance behavior is the cause for the lack of behavioral response of F1 hybrid flies in flight tunnel assays. It is possible that the disrupted olfactory system of hybrids could be a pleiotropic consequence of developmental incompatibilities due to divergent selection on other host-related traits, such as differences in the timing of diapause that adapts apple and hawthorn flies to variation in when their respective host plants fruit [27, 94, 95]. It also remains to be determined what specific aspect of the *Rhagoletis* olfactory system is impaired in hybrids. Initial studies suggested that the peripheral olfactory system was altered in hybrids, as a far higher percentage of single cell ORNs responded to multiple classes of host plant volatiles in hybrids than in parental apple and hawthorn flies [96]. This implied that these ORNs were misexpressing multiple OR genes that could disrupt behavior. However, comparison between F2 and backcross flies that both responded and failed to respond to apple and hawthorn fruit volatiles in the flight tunnel showed no differences in ORN firing patterns to specific compounds; both behavioral responders and nonresponders displayed the same altered ORN patterns that F1 hybrids did [97]. Thus, altered OR gene expression could be incidental or subtly contribute to loss of behavioral orientation in F1 hybrids but may not be the only or prime cause for the disruption. In a similar manner, altered ORN patterns do not distinguish whether habitat avoidance or a pleiotropic effect of a more general developmental incompatibility forms the basis for loss of behavior in hybrids. Additional work needs to be done to clarify these issues.

6. Additional Evidence and Problems

To determine how general habitat avoidance may be and how common disrupted host choice behavior may be in hybrids, we surveyed the plant-associated insect literature for potential examples. Although not exhaustive, we found 10 examples consistent with avoidance behavior (Table 3). These include insects across several orders, including Coleoptera, Hymenoptera, Hemiptera, and Diptera. A Y-tube or alternative olfactometer apparatus is often the method used to assess avoidance, although no-choice field and laboratory-based
tests have been used, as well. In many cases, electroantennal readings were made in addition to behavioral tests to determine whether nonhost volatiles induced electrophysiological activity. In all cases, experiments were designed to explicitly test for avoidance behavior.

A difficulty with assessing avoidance behavior stems from the nature of the experimental assays of behavior. A particular problem is that host studies are often done in choice experiments in which insects are given plant material from alternative hosts in a confined area and the relative proportions of time they choose to use the plants used as a measure of preference. The difficulty here can be twofold. First, as discussed previously, insects usually do not have to directly choose between alternate hosts in nature. Rather, more often than not, host use comes down to acceptance or rejection of a given plant. Hence, no choice experiments are a better test for preference behavior, providing that the subjects are not overly stressed by their physiological condition or circumstances to accept lower ranking hosts, regardless of plant quality. This may be especially important when testing for host avoidance. Having plants too close together in an arena in a choice test can result in mixed sensory cues confounding insects with signals not often experienced in the wild. For example, in *Rhagoletis*, the addition of nonnatal compounds to a natal fruit volatile blend (apple or hawthorn) does not just result in behavioral indifference but often an active avoidance of apple and hawthorn flies to the mixed blend [64, 65]. In choice situations between natal versus nonnatal blends, overall capture rates of apple and hawthorn flies can fall and, of great importance, discrimination for a fly’s natal blend declines [98]. It is likely that in these instances the one meter distance between natal versus nonnatal blends in these trials was not sufficient to preclude volatile plume mixing. Flies therefore interpreted both volatile blends as nonnatal and displayed decreased orientation to the natal versus nonnatal blend than in tests conducted using the natal blend versus a blank control. One must therefore be careful in interpreting the results from choice studies.

The choice trials for *Rhagoletis* also highlight a second problem in testing for avoidance behavior: the use of proportional host acceptance rather than absolute values to assess discrimination. If one were to analyze the relative proportions of acceptance of natal versus nonnatal volatiles as a comparative metric in host studies, it could appear that host discrimination goes down in multiple choice experiments versus no choice tests. The key is to estimate and compare absolute acceptance behaviors in no choice trials, however, as well as the time it takes for acceptance. If not, evidence for host avoidance could be masked and underestimated. For example, it may be that 50% of the time a hybrid insect may choose one plant versus another in a choice test, suggesting no preference or avoidance on a relative scale. However, it may be that hybrids come to reside on plants and less often than their parents do. Thus, although the hybrids are not showing a preference difference, on an absolute scale they are making host acceptance decisions less often than parental individuals, which could reflect the existence of conflicting avoidance behavior.

We also surveyed the literature for plant-associated insects to look for behavioral host choice dysfunction in hybrids consistent with avoidance alleles reducing hybrid fitness (Table 4). Here, the current evidence mainly comes from *Rhagoletis*, a leaf-feeding beetle (*Neochlamisus bebbianae*) and a hymenopteran parasitoid (*Leptopilina boulardi*). In all cases, F1 hybrids express a reduced ability to orient or respond to parental host plants or host-plant volatiles.

### 7. New Empirical Data

As an example of how one might implement a test for habitat avoidance, we recently conducted a preliminary Y-tube olfactory study testing for nonnatal host avoidance for a braconid parasitoid, *Utetes lectoides*, attacking the fruit fly *Rhagoletis zephyria* in snowberry fruit in the western USA. Domesticated apples were brought by settlers to the Pacific Northwest region of the USA in the last 200 years and it is believed that *R. pomonella* was introduced via larval

<table>
<thead>
<tr>
<th>Species</th>
<th>Order; Family</th>
<th>Assessment method(s)</th>
<th>Electrophysio. response nonhost volatiles?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dryocosmus kuriphilus</em></td>
<td>Hymenoptera: Cynipidae</td>
<td>Y-tube olfactometer</td>
<td>Yes</td>
<td>[61]</td>
</tr>
<tr>
<td><em>Sitophilus zeama</em></td>
<td>Coleoptera: Curculionidae</td>
<td>Four-way olfactometer</td>
<td>?</td>
<td>[62]</td>
</tr>
<tr>
<td><em>Brevicoryne brassicae</em></td>
<td>Hemiptera: Aphididae</td>
<td>Y-tube olfactometer</td>
<td>Yes</td>
<td>[63]</td>
</tr>
<tr>
<td><em>Aphis fabae</em></td>
<td>Hemiptera: Aphididae</td>
<td>Y-tube olfactometer</td>
<td>Yes</td>
<td>[63]</td>
</tr>
<tr>
<td><em>Rhagoletis pomonella</em></td>
<td>Diptera: Tephritidae</td>
<td>Field test; no-choice flight tunnel</td>
<td>Yes</td>
<td>[64, 65]</td>
</tr>
<tr>
<td><em>Diachasma alloemum</em></td>
<td>Hymenoptera: Braconidae</td>
<td>Y-tube olfactometer</td>
<td>Yes</td>
<td>[66]</td>
</tr>
<tr>
<td><em>Pityogenes chalcographus</em></td>
<td>Coleoptera: Curculionidae</td>
<td>Field test</td>
<td>?</td>
<td>[67]</td>
</tr>
<tr>
<td><em>Tomicus piniperda</em></td>
<td>Coleoptera: Curculionidae</td>
<td>Field test</td>
<td>Yes</td>
<td>[68]</td>
</tr>
<tr>
<td><em>Hylurgops palliatus</em></td>
<td>Coleoptera: Curculionidae</td>
<td>Field test</td>
<td>?</td>
<td>[68]</td>
</tr>
<tr>
<td><em>Heliothrips haemorrhoidalis</em></td>
<td>Thysanoptera: Thripidae</td>
<td>unknown</td>
<td>?</td>
<td>[70]</td>
</tr>
</tbody>
</table>
infested apples within the last 50 years. We hypothesized that snowberry fly origin populations of *U. lectoides* in the western USA might have recently evolved to avoid the volatile odors emanating from introduced apple fruit. A glass Y-tube with each arm connected to a filtered air source was set on a flat surface with an attractive light source at the end of the tube where the arms intersect. All wasps were individually tested for behavioral orientation in the system by assessing whether they turn into the left-hand arm, or the right-hand arm, or whether they fail to reach the y-intersection. This establishes a baseline level of response due to the system itself, in the absence of fruit volatiles. An odor (here, emitted from the surface of apple or snowberry fruit) can then be added to just one randomly selected arm of the tube, and wasps reintroduced to the system. Avoidance (or preference) is measured by the change in response to the arm of the tube containing the odor. Preliminary results for *U. lectoides* were consistent with the nonnatal habitat avoidance hypothesis. Only 1 of 12 wasps attacking snowberry wasps that were tested (8.3%) oriented to the arm of the Y-tube containing apple fruit volatiles versus a blank, odorless arm compared to the established 42% baseline control response when both apple fruit volatiles versus a blank, odorless arm compared to the established 42% baseline control response when both arms were blank ($\chi^2 = 4.9, P = 0.026, 1$ df). Additional testing with larger sample sizes is needed to confirm the pilot study results and to better establish the extent to which *U. lectoides* orients to snowberry volatiles (4 of 9 = 44% of wasps did in our initial study). Nevertheless, this example demonstrates avoidance and how one might easily test for such behavior.

### 8. Conclusions

In this paper, we have investigated the idea that avoidance behavior may play a significant role in how organisms select the habitats they reside in (see also [99] for a complementary review of habitat choice). We contend that in addition to positively orienting to certain critical cues in their natal habitat, organisms also actively reject alternative habitats that contain nonnatal elements. If true, then the evolution of avoidance behavior can have important implications for the evolution of ecologically based reproductive isolation that go beyond the pre-zygotic barriers resulting from preference alone. Specifically, contrasting avoidance behaviors can cause host choice conflicts in hybrids, resulting in postzygotic reproductive isolation. Similar thinking about behavioral avoidance could also apply to sexual selection and reinforcement, widening the consequences of these processes for postzygotic isolation during speciation.

We discussed the current theoretical and empirical evidence for habitat avoidance, focusing on phytophagous insects as a model system. In general there is evidence supporting habitat avoidance, but more work needs to be done to verify that avoidance conflicts in hybrids directly cause F1 inviability and sterility in finding habitats and mates. In allopatry, there would seem to be no theoretical difficulty for selection on habitat choice to generate behavioral, as well as developmental conflicts, in hybrids following secondary contact. Although more difficult in sympatry or parapatry, we outlined how it is also possible to evolve habitat avoidance that causes postzygotic isolation in the face of gene flow. More theoretical work needs to be done in this area to produce estimates of how probable it is for new mutations having differing effects on avoidance and behavioral incompatibility to establish between diverging populations. Experimental work also clearly shows that insects use certain chemical cues from nonhosts to avoid these plants. However, it must still be clearly shown that reduced host choice response in hybrids is due to conflicting avoidance behaviors rather than to the pleiotropic consequences of developmental incompatibilities for other traits affecting sensory systems involved in habitat choice. Moreover, detailed neural physiology and genetic studies are needed to determine and map how avoidance evolves and how habitat choice is disrupted in hybrids. Analysis of habitat choice, and avoidance behavior in particular, is still in its early stages but is an intriguing area of theoretical and empirical study linking ecology adaptation and speciation with physiology, development, and genetics. Our current

### Table 4: Studies presenting data consistent with habitat avoidance alleles causing problems in hybrids. Each cross-generated hybrids displaying reduced response relative to parents in habitat choice. Host plant or locality information is in parentheses.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Hybrid cross</th>
<th>Reduced hybrid response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhagoletis fruit flies</td>
<td><em>R. pomonella</em> (apple) × <em>R. pomonella</em> (haw)</td>
<td>Upwind flight to host odor</td>
<td>[82]</td>
</tr>
<tr>
<td>Rhagoletis fruit flies</td>
<td><em>R. pomonella</em> (apple) × <em>R. nr. pomonella</em> (dogwood)</td>
<td>Upwind flight to host odor</td>
<td>[82]</td>
</tr>
<tr>
<td>Rhagoletis fruit flies</td>
<td><em>R. pomonella</em> (haw) × <em>R. nr. pomonella</em> (dogwood)</td>
<td>Upwind flight to host odor</td>
<td>[82]</td>
</tr>
<tr>
<td>Rhagoletis fruit flies</td>
<td><em>R. pomonella</em> (apple) × <em>R. mendax</em> (blueberry)</td>
<td>Electroantennogram to fruit odor</td>
<td>[43]</td>
</tr>
<tr>
<td>Neoclamisus leaf beetles</td>
<td><em>N. bebbiana</em> (maple) × <em>N. bebbiana</em> (willow)</td>
<td>Time spent on foraging on parental host plants</td>
<td>Egan and Funk, unpubl.; [83]</td>
</tr>
<tr>
<td>Leptopilina parasitoids</td>
<td><em>L. boulardi</em> (Nasrallah) × <em>L. boulardi</em> (Brazzaville)</td>
<td>Probing response to fruit odor $^1$</td>
<td>[84]</td>
</tr>
</tbody>
</table>

$^1$ Only in one direction of the F1 hybrid cross (Nasrallah female × Brazzaville male).
knowledge, while incomplete, suggests that there may be great ecological significance and evolutionary potential for the often anthropomorphically ill-viewed trait of disdain.

Acknowledgments

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References


Larval Performance in the Context of Ecological Diversification and Speciation in *Lycaeides* Butterflies

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The role of ecology in diversification has been widely investigated, though few groups have been studied in enough detail to allow comparisons of different ecological traits that potentially contribute to reproductive isolation. We investigated larval performance within a species complex of *Lycaeides* butterflies. Caterpillars from seven populations were reared on five host plants, asking if host-specific, adaptive larval traits exist. We found large differences in performance across plants and fewer differences among populations. The patterns of performance are complex and suggest both conserved traits (i.e., plant effects across populations) and more recent dynamics of local adaptation, in particular for *L. melissa* that has colonized an exotic host. We did not find a relationship between oviposition preference and larval performance, suggesting that preference did not evolve to match performance. Finally, we put larval performance within the context of several other traits that might contribute to ecologically based reproductive isolation in the *Lycaeides* complex. This larger context, involving multiple ecological and behavioral traits, highlights the complexity of ecological diversification and emphasizes the need for detailed studies on the strength of putative barriers to gene flow in order to fully understand the process of ecological speciation.

1. Introduction

Understanding the processes underlying diversification is a central question in evolutionary biology. Lineages diversify along multiple axes of variation, including morphological, physiological, and ecological traits. With respect to diversification in ecological traits, many recent studies have found that ecological niches can be highly conserved from a macroevolutionary perspective [1–3]. In other words, closely related species tend to utilize similar resources or occupy similar environments. In contrast, the field of ecological speciation suggests that ecological traits can evolve due to disruptive selection and drive the process of diversification [4–8]. In herbivorous insects, evolution in response to habitat or host shifts is often thought to be a first step in the evolution of reproductive isolation [9, 10]. In most well-studied systems, although exceptions exist [6, 11], niche conservatism and niche evolution are often characterized by a small number of ecological traits, such as habitat preference or physiological performance [12–14]. To understand the causes and consequences of evolution in ecological traits, more studies are needed of groups in which diversification is recent or ongoing and multiple ecological traits are studied. The study of multiple traits is particularly important for our understanding of ecological speciation. For example, it has been suggested that weak selection acting on a multifarious suite of traits could be as important for speciation as strong selection acting on a single ecological trait [15].

The butterfly genus *Lycaeides* (Lycaenidae) includes a complex of taxa in North America that has been the focus of studies investigating the evolution and ecology of host use, mate choice, and genital morphology, among other
In the context of diversification, this group is interesting because hybridization has been documented among multiple entities, with a variety of consequences [20, 21], including the formation of at least one hybrid species in the alpine of the Sierra Nevada mountains [22]. The *Lycaeides* taxa in western North America (specifically *L. idas*, *L. melissa*, and the hybrid species) differ in many traits, some of which have been implicated in the evolution of ecological reproductive isolation in this system. For example, there is variation in the strength of host preference, which is often linked to reproductive isolation in herbivorous insects that mate on or near their host plants, as *Lycaeides* do [17]. There are also potentially important differences in mate preference, phenology, and egg adhesion [16, 23]. The latter trait is interesting with respect to the evolution of the hybrid species, which lacks egg adhesion [22, 23]. The eggs of the hybrid species fall from the host plants. This is presumed to be an adaptation to the characteristics of the alpine plants, for which the above-ground portions senece and are blown by the wind away from the site of next year’s fresh growth (thus eggs that fall off are well positioned for feeding in the spring). Since the eggs of lower-elevation *Lycaeides* taxa do adhere to hosts, this trait could serve as a barrier to gene flow with respect to individuals immigrating from lower elevations.

Our state of knowledge for *Lycaeides* is unusual for well-studied groups of herbivorous insects in that we know a great deal about a diversity of traits, as discussed above, but we have not heretofore investigated larval performance across taxa in the context of ecological speciation, which is often one of the first traits studied in other insect groups [24]. This study has two goals, first to investigate larval performance and then to put this information in the context of other already-studied traits to investigate which traits might be important for reducing gene flow between populations and species in this system. We have focused on performance of caterpillars from both *L. idas* and *L. melissa* populations as well as from populations of the hybrid species. Beyond the inclusion of the hybrid taxon, of added interest is the fact that *L. melissa* has undergone a recent expansion of diet, encompassing exotic alfalfa (*Medicago sativa*) as a larval host plant across much of its range. Thus we are able to investigate variation in the key ecological trait of larval performance across multiple levels of diversification, including the differentiation of *L. idas* and *L. melissa*, the formation of a hybrid species, and a host expansion that has occurred within the last two hundred years [19, 22, 25].

Using individuals from two *L. idas*, three *L. melissa*, and two hybrid species populations, we conducted reciprocal rearing experiments using all five of the host species found at these focal populations. We assessed larval performance by examining survival, time to emergence (eclosion), and adult weight, and by comparing survival curves from different populations on the different plants. For each population, we contrasted larval performance on a natal host to performance on the plants of other populations. Higher larval performance on natal host plants would support the hypothesis of local adaptation to host plant species. In the second part of the paper, these results are discussed both within the light of local adaptation in a diversifying group and also within the context of possible reproductive isolation related to variation in ecological traits.

### 2. Methods

Two of our focal taxa, *L. idas* and *L. melissa*, are widely distributed across western North America. Our study focused on populations of these two species and the hybrid species in northern California and Nevada (Figure 1). In this area, *L. idas* is found on the west slope of the Sierra Nevada, *L. melissa* is found on the eastern side, and the hybrid species is only found in the alpine zone. *Lycaeides* species use a variety of plants in the pea family, Fabaceae, as hosts, although (with few exceptions) specific *Lycaeides* populations generally utilize a single host plant species. The two *L. idas* populations studied were Yuba Gap (YG), which uses *Lotus nevadensis* as a host, and Leek Springs (LS) which uses the host *Lupinus polyphyllus* (Table 1). Both populations of the hybrid species, Mt. Rose (MR) and Carson Pass (CP), use *Astragalus whitneyi*. At Washoe Lake (WL), *L. melissa* uses the native host *Astragalus canadensis*; at Beckworth Pass (BP), butterflies use both *A. canadensis* and alfalfa (*Medicago sativa*); at Goose Lake (GLA), the only available host is alfalfa (Table 1).

*Lycaeides idas* and the hybrid species are univoltine, while *L. melissa* populations have at least three generations per year. Eggs from the univoltine populations have to be maintained under winter conditions (i.e., cold temperatures and darkness) in the lab for experiments in the following spring. Females and eggs from univoltine populations were collected in the summer of 2008 to be reared in the summer of 2009, while *L. melissa* females and eggs were collected during the 2009 summer. Females were collected from the *L. idas* and hybrid species populations (32 from Yuba Gap, 50 from Leek Springs, 45 from Carson Pass, and 40 from Mt. Rose) and caged individually or in small groups with host plants for a period of three days after which eggs were collected. Eggs were washed with a dilute (2%) bleach solution and held over the winter at 4–6°C. Eggs were removed from cold storage on May 27th, 2009, and the majority hatched within several hours. The number of caterpillars hatching synchronously required that the larvae be moved in groups of twenty to standard-sized petri dishes (100 mm diameter) with fresh plant material on the 27th and 28th. On the 29th and 30th of May, the groups of twenty were split into three dishes each containing three to seven individuals. An average of 6 caterpillars was added to 9 dishes per treatment (plant/population combination); in some cases fewer (but not less than three) caterpillars were added per dish to try to maximize the number of dishes, which is the unit of replication (see below). Once all the larvae in a petri dish reached the 3rd or 4th instar they were moved to larger petri dishes (170 mm diameter). These groups of individuals were considered a “rearing dish,” and dish was used as a random factor in statistical analyses (see below).

Females and eggs from the three *L. melissa* populations were collected following similar protocols (though without the necessity of overwintering). Seventeen females were
L. idas

Yuba Gap (YG)
Carson Pass (CP)
Mt. Rose (MR)
Washoe Lake (WL)
Beckwourth Pass (BP)
Goose Lake (GLA)

L. melissa

Alpine

Table 1: Locations of populations (see also Figure 1) and hosts associated with the seven populations studied.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Location</th>
<th>Latitude/longitude</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. idas</td>
<td>Leek Springs (LS)</td>
<td>38°38′8″ N/120°14′25″ W</td>
<td>Lupinus polyphyllus</td>
</tr>
<tr>
<td></td>
<td>Yuba Gap (YG)</td>
<td>39°19′24″ N/120°35′60″ W</td>
<td>Lotus nevadensis</td>
</tr>
<tr>
<td>Hybrid species</td>
<td>Carson Pass (CP)</td>
<td>38°42′28″ N/120°0′28″ W</td>
<td>Astragalus whitneyi</td>
</tr>
<tr>
<td></td>
<td>Mt. Rose (MR)</td>
<td>39°19′21″ N/119°55′47″ W</td>
<td>Astragalus whitneyi</td>
</tr>
<tr>
<td></td>
<td>Washoe Lake (WL)</td>
<td>39°13′59″ N/119°46′46″ W</td>
<td>Astragalus canadensis</td>
</tr>
<tr>
<td>L. melissa</td>
<td>Beckwourth Pass (BP)</td>
<td>39°46′55″ N/120°4′23″ W</td>
<td>Astragalus canadensis and Medicago sativa</td>
</tr>
<tr>
<td></td>
<td>Goose Lake (GLA)</td>
<td>41°59′9″ N/120°17′32″ W</td>
<td>Medicago sativa</td>
</tr>
</tbody>
</table>

Larvae from each population were reared on all five plants, Astragalus canadensis, Astragalus whitneyi, Lotus nevadensis, Lupinus polyphyllus, and Medicago sativa, with individual rearing dishes being assigned exclusively to a single plant throughout development. Caterpillars in the wild consume both vegetative and reproductive tissues, but only leaves were used in this study, as flowers would be difficult to standardize across plants (not being available synchronously.
for most species). For a study of this kind, ideally all plant material to be used in rearing would be collected from focal locations (where butterflies are flying) or grown in a common environment. However, many of these species are not easily propagated, and moreover our focal locations are widely dispersed geographically; these factors necessitated some compromise in collecting some of the plants. *A. canadensis* cuttings were obtained at the site of the butterfly populations at Beckworth Pass and Washoe Lake and from the greenhouse (plants were grown from seeds collected at Washoe Lake). *Astragalus whitneyi* was collected from the site of the Mt. Rose hybrid species population and on a hillside adjacent to Carson Pass (38°42′23″/120°00′23″). All *Lotus nevadensis* were collected from Yuba Gap (YG). The only case in which plant material was collected from a site where the butterfly is not found is *Lupinus polyphyllus*. These plants were collected off I-80 at the Soda Springs exit (39°19′29″/120°23′25″) and seven miles north of Truckee CA, off State Route 89 (39°25′59″/120°12′13″). *Medicago sativa* was obtained from Beckworth Pass (BP) and from plants grown in the greenhouse with seeds from BP. *M. sativa* was also collected from south of Minden, NV on State Route 88 (38°48′60″/119°46′46″) and off of California State Route 49 in Sierra Valley, CA (39°38′35″/120°23′10″). Plant material was kept in a refrigerator and larvae were fed fresh cuttings whenever the plant material in petri dishes was significantly reduced or wilted, which was approximately every two to seven days. Each time caterpillars were given fresh plant material, the number of surviving caterpillars was recorded along with the date. All dishes were kept at room temperature, 20° to 23° Celsius, on lab benches. Newly emerged adults were individually weighed to the nearest 0.01 mg on a Mettler Toledo XP26 microbalance and sex was recorded.

2.1. Analyses. The strengths of our experiment were that we reared a large number of individuals from multiple taxa across five plants, but a weakness of our design was that not all rearing could be done simultaneously. As discussed further below, flowers were not included in the rearing, and plant material was collected from most but not all focal populations. Experiments were conducted in two phases, first involving the populations of the hybrid species and *L. idas*, being reared together and earlier in the spring, and second involving the three low-elevation *L. melissa* populations being reared later in the summer. This division into two rearing groups was largely a consequence of being constrained by the total number of caterpillars that could be handled and reared in the lab at any one time. Considering the possibility that phenological variation in plants could have implications for larval performance, we conducted analyses separately for the three butterfly species. Postemergence adult weight, time to emergence as adult, and survival to adult were recorded. Mortality (reflected in the survival data) included death associated with caterpillars that died while developing, individuals that pupated but failed to emerge, and disease; we did not distinguish between these sources of mortality. Data were standardized (Z transformed) within populations to facilitate comparisons among populations and taxa that may have inherent differences, such as in size or in development time. Z scores were used in analyses described below unless otherwise noted.

Dish was considered the unit of replication, thus percent survival was calculated per dish. For analyses of adult weight and time to emergence, dish was used as a random factor. Percent survival was analyzed using analysis of variance (ANOVA) with plant, population, and the interaction between the two as predictor variables. Time to emergence and adult weight were both analyzed with ANOVA, using population, plant, the interaction between the two and sex as predictor variables, along with dish as a random factor nested within plant and population. For all of these analyses, ANOVA was performed a second time without the plant/population interaction if it was not significant at α < 0.05. These analyses were performed using JMP software version 8.0.2 (SAS Institute).

Differences in survival were also investigated by generating and comparing survival curves. To create survival curves, individual caterpillars were assumed to be alive until the date they were found dead. Rather than analyzing survival curves on an individual-dish basis (where sample sizes were small), the number of individuals surviving on a given day was calculated for each plant/population combination, giving one curve per combination, as is often done in survival analysis [26]. Survival curves were generated in R (2.12.2) using the packages *splines* and *survival*. The shapes of the curves were investigated within population using the packages *MASS* and *fitdistrplus*. Weibull distributions are commonly used to model survival using two parameters, shape and scale. The shape parameter measures where the inflection point occurs or practically whether individuals are lost more at the beginning or end of a given time period, and the scale parameter characterizes the depth of the curve. We estimated the two Weibull parameters, shape and scale, that characterized the fitted curves using maximum likelihood. One-thousand bootstrap replicates were then used to generate 95% confidence intervals for the shape and scale parameters, so that they could be compared across plants within a given population.

3. Results

We began the larval performance experiments with 2040 caterpillars in 357 dishes. Average survival to eclosion across all experiments was 23.4%. In general, differences in larval performance among plants were greater than differences between populations, which can be seen both in Figure 2 and also by comparing variation partitioned by plants and population in Table 2. For example, survival was highest on *Lotus nevadensis* across all populations for all three taxa, with an average survival of 56.5% (survival on *Lupinus polyphyllus* was comparable for two of the three *L. melissa* populations). Survival on alfalfa was consistently the lowest of any plant across populations: only two caterpillars survived to eclosion (Figures 2 and 3). Because survival was so low on alfalfa, it was excluded from most analyses and figures. The inferior nature of alfalfa as a host plant is consistent with previous studies, particularly when caterpillars do not have access to...
flowers and flower buds. When flowers have been included in performance experiments, survival of L. melissa on alfalfa and A. canadensis was equal, although those individuals reared on alfalfa were significantly smaller adults [19].

Plant and the interaction between plant and population were significant predictors of survival for L. idas, L. melissa, and the hybrid species (Table 2). Within taxa, there were differences among populations on certain plants. For example, survival for L. idas on Lotus nevadensis was greater for LS compared to YG, but the pattern was reversed for the host Lupinus polyphyllus (Figure 2); in other words, each population had higher survival on the natal host of the other population. A different pattern can be seen across populations of L. melissa on Astragalus canadensis, where survival was highest for individuals from WL, a population whose natal host is A. canadensis. L. melissa survival on A. canadensis was lowest for GLA, which is a population associated with the exotic host alfalfa, and survival on A. canadensis is intermediate for BP, where both A. canadensis and alfalfa are utilized. Thus host use by L. melissa populations predicts variation in larval performance. Effects of plant and population were generally not as pronounced for either adult weight or time to emergence (for L. idas, the only significant predictors of adult weight were dish and sex); exceptions to this include the significant population by plant interaction for adult weight of L. melissa. As with survival, L. melissa performance (adult weight) was greater on A. canadensis for the population that is associated with that plant, WL (Figure 2(f)).

Consistent with results for survival to emergence as an adult, survival curves through time also showed pronounced differences among plants (Figure 3; Table 4). For example, the Weibull scale parameter for alfalfa was generally different compared to the other plants, reflecting early and pervasive mortality for individuals reared on that plant. Most but not
Table 2: Results from analyses of variance for the three measures of performance: percent survival, adult weight, and time to emergence. In all cases dish was used as the unit of replication. Most population/plant combinations had 9 dishes, except for the following: YG/Ac 12 dishes, YG/Ln 12 dishes, YG/Lp 12 dishes, YG/Ms 12 dishes, and all plant combinations for CP and MR had 12 dishes. The total number of dishes was 357.

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>F Ratio,df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Survival L. idas</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Plant</td>
<td>48.62</td>
<td>42.02,73</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Population</td>
<td>0.02</td>
<td>0.06,73</td>
<td>0.81</td>
</tr>
<tr>
<td>Plant × population</td>
<td>4.00</td>
<td>3.46,73</td>
<td>0.02</td>
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<td><strong>Survival hybrid species</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Plant</td>
<td>30.93</td>
<td>16.33,88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Population</td>
<td>0.00</td>
<td>0.00,88</td>
<td>1.00</td>
</tr>
<tr>
<td>Plant × population</td>
<td>7.50</td>
<td>3.96,88</td>
<td>0.01</td>
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<tr>
<td><strong>Survival L. melissa</strong></td>
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<td>44.95</td>
<td>29.32,96</td>
<td>&lt;0.0001</td>
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<td>0.03,96</td>
<td>0.97</td>
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<td>Plant × population</td>
<td>9.99</td>
<td>3.26,96</td>
<td>0.006</td>
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<td><strong>Adult weight L. idas</strong></td>
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<td>Plant</td>
<td>2.11</td>
<td>0.52,33,47</td>
<td>0.67</td>
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<td>Population</td>
<td>0.49</td>
<td>0.14,19,38</td>
<td>0.71</td>
</tr>
<tr>
<td>Dish (plant, population)</td>
<td>47.78</td>
<td>4.04,64,74</td>
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<td>Sex</td>
<td>10.81</td>
<td>11.87,74,00</td>
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<td><strong>Adult weight hybrid species</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Plant</td>
<td>7.25</td>
<td>2.81,48,09</td>
<td>0.049</td>
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<td>Population</td>
<td>0.53</td>
<td>0.61,48,45</td>
<td>0.44</td>
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<td>Dish (plant, population)</td>
<td>14.90</td>
<td>0.74,30,00</td>
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<tr>
<td>Sex</td>
<td>1.29</td>
<td>1.28,30,00</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Adult weight L. melissa</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Plant</td>
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<td>4.54,154,10</td>
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<tr>
<td>Dish (plant, population)</td>
<td>89.56</td>
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<td>0.02</td>
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<tr>
<td>Sex</td>
<td>11.24</td>
<td>15.15,217,00</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Time to emergence L. idas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant</td>
<td>20.32</td>
<td>8.91,35,17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Population</td>
<td>0.69</td>
<td>0.99,14,11</td>
<td>0.37</td>
</tr>
<tr>
<td>Dish (plant, population)</td>
<td>19.34</td>
<td>0.94,28,75</td>
<td>0.55</td>
</tr>
<tr>
<td>Sex</td>
<td>3.87</td>
<td>2.45,75,00</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Time to emergence hybrid species</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Plant</td>
<td>5.30</td>
<td>3.28,25,82</td>
<td>0.05</td>
</tr>
<tr>
<td>Population</td>
<td>0.00</td>
<td>0.00,28,00</td>
<td>1.00</td>
</tr>
<tr>
<td>Dish (plant, population)</td>
<td>16.06</td>
<td>0.95,28,00</td>
<td>0.54</td>
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<tr>
<td>Sex</td>
<td>1.20</td>
<td>1.41,28,00</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Time to emergence L. melissa</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant</td>
<td>9.36</td>
<td>2.41,106,23</td>
<td>0.07</td>
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<tr>
<td>Population</td>
<td>1.66</td>
<td>0.61,99,01</td>
<td>0.54</td>
</tr>
<tr>
<td>Dish (plant, population)</td>
<td>125.50</td>
<td>2.34,422,00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex</td>
<td>24.18</td>
<td>37.87,223,00</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Figure 3: Continued.
all mortality was manifested quite early in development, particularly for the alpine and *L. idas* populations across plants. Mortality was more evenly distributed through time for *L. melissa* on all plants. In some cases, patterns of survival vary among plants within populations, even when overall survival was low. For example, survival curves for CP drop much less rapidly for three plants, one of which is the natal host *A. whitneyi* and another is the congeneric *A. canadensis* (the third is *L. nevadensis*). For *A. canadensis*, it is interesting to note that across all populations there was a drop-off in survival near the end of development: many individuals made it to the pupal stage, but failed to emerge, perhaps suggesting a subtle nutritional challenge for successful completion of development presented by that plant.

### 4. Discussion

The reciprocal rearing experiment detected strong host plant effects and limited evidence of local adaptation to natal host plant species in the *Lycaeides* species complex. For example, development on *L. nevadensis* (the host of the *L. idas* population at YG) resulted in relatively high survival throughout the experiment, while development on *Medicago sativa* (the exotic host of *L. melissa* at GLA and BP) led to extremely low survival in all cases. These plant effects that transcend populations could be indicative of larval traits (such as high survival on *L. nevadensis*) that are conserved in the group and are not particularly labile. Our results could be influenced by the use of leaves but not flowers in larval rearing. Previous work has shown that survival is improved on *Medicago sativa* for larvae that have access to flowers, but this is not true on *Astragalus canadensis* [19]. We do not know if flowers are or are not important for larvae developing on the other plants.

In general, the survival that we report (23.4% throughout the experiment) could reflect the absence of flowers or other unfavorable lab conditions, and we do not at this time have life history data from the field for *Lycaeides* with which to compare our results. However, in interpreting results here and elsewhere (e.g., [19]), we make the assumption that lab experiments are informative with respect to relative performance across hosts. In other words, the consumption of *M. sativa* by *Lycaeides* caterpillars is associated with development into adults that are small relative to adults that develop on other plants. Without artifacts of lab rearing, it is possible that performance would generally be higher in the wild, but we would predict that performance on *M. sativa* would still be lower relative to performance on native hosts. An alternate possibility, which we cannot test at this time, is that lab rearing has plant-specific effects (i.e., *M. sativa* is a poor host only when used under artificial conditions).

For all the performance results, it is also important to note that phenological effects of changes in plant quality or suitability could be pronounced, but are not addressed by our experimental design. In particular, as noted above, our rearings were conducted in two phases due to logistical constraints: first including *L. idas* and populations of the hybrid species and second including all three *L. melissa* populations. This is not a completely unnatural situation, as *L. idas* and the hybrid species are univoltine, while *L. melissa* populations are multivoltine. Thus *L. idas* and hybrid species caterpillars are more likely to be exposed to only the early spring vegetation, as in our experiment. The consideration of phenological effects in plants is most relevant when comparing performance among butterfly taxa (e.g., the performance of *L. melissa* versus *L. idas* on a particular plant) but is less important when making comparisons within a taxon (e.g., the performance of *L. melissa* on different plant species).

**Figure 3:** Survival curves for the seven populations studied. Colors indicate survival associated with a given plant; letters next to each plant in legends correspond to differences in the shape (upper case) and scale (lower case) of each curve indicated by nonoverlapping 95% confidence intervals from bootstrapped parameter values (see Table 4 for more details). Black dots indicate native host association for each population. Average, final survival is shown to the right of each graph for nonzero results. Plant abbreviations as follows. *Ac*; *Astragalus canadensis*; *Aw*; *Astragalus whitneyi*; *Ln*; *Lotus nevadensis*; *Lp*; *Lupinus polyphyllus*; *Ms*; *Medicago sativa*.
In contrast to the general result of strong plant effects across taxa and populations, one result suggestive of local adaptation is the performance (survival and adult weight) of L. melissa on the native host A. canadensis [27]. Performance was highest on the native for the population that utilizes that host in the wild and lowest for a population associated with the exotic host alfalfa. Performance on A. canadensis is intermediate for the population where both hosts are used. These results raise a number of possibilities, including a scenario in which genetic variants associated with higher performance on an ancestral host were lost in the transition to the exotic host, which could be a consequence of relaxed selection or a population bottleneck in the new environment. Another explanation could involve a change in gene regulation associated with performance, rather than a loss of alleles. In any event, the transition to the novel host has apparently not been accompanied by an increase in performance on alfalfa. One caveat to this conclusion is that the M. sativa used in experiments was collected at one of the focal locations (BP), but could not, for logistical reasons, be collected from GLA. The latter population (GLA) is the population associated only with M. sativa, thus the conclusion that performance has not increased following the colonization of the novel host could have been different if local plant material from that location had been used in experiments; however, we have found consistently low performance on M. sativa in other experiments [19], suggesting generality to the result of low performance on that plant.

Variation in host preference has previously been documented among populations of Lycaeides butterflies, with populations of the hybrid species in particular exhibiting strong preferences for their natal host, A. whitneyi [17, 22], relative to the hosts of other Lycaeides populations. However, we found low survival and low adult weights for individuals of the hybrid species reared on A. whitneyi (Figure 2). It is possible that laboratory conditions were a poor reflection of appropriate abiotic conditions for the hybrid species individuals adapted to an alpine environment. It is also possible that other factors, such as the absence of flowers in experiments or induced defenses in leaves, could be important in A. whitneyi, which supported poor growth for larvae from all populations. In any event, the patterns of performance that we report are not consistent with an expected preference-performance paradigm for host shifts leading to the evolution of reproductive isolation [28]. Variation in both adult preference and larval performance is discussed further in the following section considering ecological traits and hypotheses relating traits to reproductive isolation.

4.1. Ecology and Diversification. Although many studies of herbivorous insects have focused on larval performance with respect to local adaptation and ecological speciation, populations of herbivorous insects (or of any organism) can of course differ in numerous ways, some related to resource use but also to other aspects of the environment. Nosil et al. [15] have suggested a number of scenarios in which multiple traits could be important in the evolution of reproductive isolation. In particular, natural selection acting on a single ecological trait or a single niche dimension could be important for initiating speciation, while the evolution of differences along multiple ecological axes might often be needed for complete reproductive isolation [15]. Multiple ecological axes could be different aspects of, for example, resource use [29, 30], or they could be more disparate traits, such as mate finding or predator avoidance. In either case, the idea is that selection along one axis might be insufficient for reproductive isolation, but selection acting along multiple axes might confer a high, overall level of reproductive isolation.

Considering the potential importance of multiple traits in ecological speciation, Figure 4 and Table 3 summarize information from this and other studies in Lycaeides and present hypotheses regarding multiple ecological and behavioral traits and how these might interact with ecological, reproductive isolating processes in this system. Specifically, Figure 4 explores hypotheses about reduced gene flow, represented by faded arrows, between the taxa due to the ecological differences of a given trait. For example, the model shown for egg adhesion posits that variation in adhesion could be a barrier to gene flow going from L. idas and L. melissa populations into populations of the hybrid species. L. idas and L. melissa females lay eggs that adhere to plants. As discussed above, the alpine host plants senesce and are blown from the area, thus removing any attached eggs from the site.

**Figure 4:** Summary of hypotheses relating ecological traits to reproductive isolation between taxa based on the current and past studies [16, 17, 19, 22, 23]. Arrows joining two taxa correspond to greater gene flow and those that are faded represent gene flow that could be prevented or reduced by a given trait. For details see text.
of next-spring’s fresh plant growth [23]. Differences in host preference might also affect patterns of gene flow between the species. The host of the hybrid species populations is readily accepted by ovipositing females from all Lycaenidae examined thus far [17], thus it would likely be accepted by females from *L. idas* and *L. melissa* populations arriving at a population of the hybrid species. In contrast, the hosts of the *L. idas* populations are not preferred by females of either the hybrid species or *L. melissa* [17, 19]. The arrows pointing towards *L. melissa* assume the presence of only the native host *A. canadensis*, not the exotic *M. sativa* (excluding the exotic is a simplifying assumption for Figure 4, but also appropriate given that ecological diversification occurred before the recent introduction of alfalfa). *Astragalus canadensis* and *A. whitneyi* are equally acceptable for oviposition by hybrid species individuals (Forister, unpublished data), and we assume the same equivalence for *L. idas* (i.e., we assume *L. idas* females would readily accept *A. canadensis*, just as they do with *A. whitneyi*, thus an arrow without a barrier pointing from *L. idas* to *L. melissa* in the host preference diagram).

Similar to host preference, variation in male mate discrimination potentially presents barriers only between hybrid species and *L. idas* populations and between *L. idas* and *L. melissa* populations but not between hybrid species and *L. melissa* populations [17]. *L. melissa* males will readily approach either *L. idas* or females of the hybrid species, while *L. idas* males discriminate against females from the other two taxa, and hybrid species males discriminate against *L. idas* females. This behavioral variation among taxa, reported in Fordyce et al. [16], comes from choice tests involving dead and paper-model females presented in experimental arrays in the field. It is important to note that being a less-preferred mate is of course not the same as not being mated. In other words, a virgin *L. melissa* female that immigrated into an *L. idas* population might be a low-ranked mate for male *L. idas* relative to local females, but it is possible that she would eventually find a mate. However, the patterns of gene flow shown in Figure 4 are meant to be hypotheses for potential barriers to gene flow within a given trait. An *L. melissa* female immigrating into a population of the hybrid species would be mated more readily (and thus be more likely to contribute to the gene pool) relative to the dynamic just described (an *L. melissa* female arriving at an *L. idas* population). It is possible to imagine all of the traits depicted in Figure 4 being involved in either pre- or postzygotic isolation. For example, mate preference could act as just described on immigrant, virgin females, as in the immigrant inviability concept of Nosil et al. (2005) [31]. An alternative but similar scenario could involve the offspring of an immigrant; in this case, wing-pattern alleles (related to mate choice) would interact with mate choice in the next generation.

Variation in voltinism could affect gene flow from *L. melissa* into the other univoltine taxa. Because *L. melissa* populations are multivoltine, it is possible that an *L. melissa* female moving into populations of the other taxa would lay eggs that failed to diapause in habitats where the univoltine strategy is superior (such as in the alpine habitat where there is a short window for larval development [32]). Alternatively, diapause could be plastic, in which case the patterns of connectivity (hypothesized patterns of gene flow) pictured would be different.

We can now add larval performance to the suite of hypotheses linking ecology and gene flow in Lycaenidae. In generating hypotheses relating larval performance to gene flow, we have used this criterion (focusing on survival, rather than adult weight, as the most straightforward metric of performance): if foreign larvae (i.e., the offspring of a recently arrived female) have lower survival on the local host relative to local individuals, we hypothesize a relative reduction in gene flow associated with performance. For gene flow between *L. idas* and *L. melissa*, the survival of *L. idas* larvae on the host of *L. melissa* is lower than the survival of *L. melissa* caterpillars on the same plant (see mean survival values in Figure 3). Interestingly in the context of hybrid speciation, our results suggest that gene flow from both *L. idas* and *L. melissa* would be unimpeded into populations of the hybrid species relative to the reverse, meaning that the two parental species had higher survival (relative to hybrid individuals) on the alpine host and that hybrid individuals had relatively inferior performance on the two parental species’ hosts. Of course, this could be different if another trait, for example, egg adhesion, had a stronger effect or

<table>
<thead>
<tr>
<th>L. idas</th>
<th>Hybrid species</th>
<th>L. melissa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg adhesion</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Preference for natal host(s)</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Male mate discrimination</td>
<td>Against <em>L. melissa</em> and hybrid species</td>
<td>Against <em>L. idas</em></td>
</tr>
<tr>
<td>Voltinism</td>
<td>Univoltine</td>
<td>Univoltine</td>
</tr>
<tr>
<td>Larval performance</td>
<td>Poor on hosts of <em>L. melissa</em>; superior on host of hybrid species</td>
<td>Poor on hosts of both <em>L. melissa</em> and <em>L. idas</em></td>
</tr>
</tbody>
</table>

Further information on specific behavioral and ecological variables (other than larval performance, reported here) can be found as follows: egg adhesion [23], preference for natal hosts [17, 19, 22], male mate discrimination [16], and voltinism [23].
Table 4: Survival curves for each host-population combination. A weibull distribution was fitted to each combination with 1000 bootstrap replicates. We report shape and scale parameters along with bootstrapped confidence intervals. Upper case and lower letters following shape and scale values correspond to 95% confidence intervals that do not overlap (upper case letters for shape and lower case letters for scale) within populations based on the thousand bootstrapped replicates. See Figure 3 for graphical representation of survival curves.

<table>
<thead>
<tr>
<th>Population</th>
<th>Host</th>
<th>Shape</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>Ac</td>
<td>1.47 (1.25–1.75)</td>
<td>B</td>
</tr>
<tr>
<td>CP</td>
<td>Aw</td>
<td>1.61 (1.35–1.97)</td>
<td>B</td>
</tr>
<tr>
<td>CP</td>
<td>Ln</td>
<td>0.82 (0.71–0.95)</td>
<td>A</td>
</tr>
<tr>
<td>CP</td>
<td>Lp</td>
<td>1.38 (1.12–2.62)</td>
<td>B</td>
</tr>
<tr>
<td>CP</td>
<td>Ms</td>
<td>1.67 (1.34–2.42)</td>
<td>B</td>
</tr>
<tr>
<td>BP</td>
<td>Ac</td>
<td>1.50 (1.17–2.01)</td>
<td>B</td>
</tr>
<tr>
<td>BP</td>
<td>Aw</td>
<td>1.31 (1.07–1.61)</td>
<td>B</td>
</tr>
<tr>
<td>BP</td>
<td>Ln</td>
<td>0.44 (0.38–0.52)</td>
<td>A</td>
</tr>
<tr>
<td>BP</td>
<td>Lp</td>
<td>0.44 (0.39–0.51)</td>
<td>A</td>
</tr>
<tr>
<td>BP</td>
<td>Ms</td>
<td>2.65 (2.00–4.07)</td>
<td>BC</td>
</tr>
<tr>
<td>GLA</td>
<td>Ac</td>
<td>2.154 (1.76–2.69)</td>
<td>B</td>
</tr>
<tr>
<td>GLA</td>
<td>Aw</td>
<td>0.88 (0.74–1.07)</td>
<td>A</td>
</tr>
<tr>
<td>GLA</td>
<td>Ln</td>
<td>0.66 (0.53–0.86)</td>
<td>A</td>
</tr>
<tr>
<td>GLA</td>
<td>Lp</td>
<td>0.94 (0.68–1.50)</td>
<td>A</td>
</tr>
<tr>
<td>GLA</td>
<td>Ms</td>
<td>1.70 (1.44–2.10)</td>
<td>AB</td>
</tr>
<tr>
<td>LS</td>
<td>Ac</td>
<td>1.57 (1.21–2.86)</td>
<td>A</td>
</tr>
<tr>
<td>LS</td>
<td>Aw</td>
<td>2.31 (1.97–3.09)</td>
<td>B</td>
</tr>
<tr>
<td>LS</td>
<td>Ln</td>
<td>0.88 (0.67–1.24)</td>
<td>A</td>
</tr>
<tr>
<td>LS</td>
<td>Ms</td>
<td>2.70 (2.20–3.70)</td>
<td>B</td>
</tr>
<tr>
<td>MR</td>
<td>Ac</td>
<td>1.11 (0.97–1.23)</td>
<td>A</td>
</tr>
<tr>
<td>MR</td>
<td>Aw</td>
<td>1.84 (1.59–2.16)</td>
<td>B</td>
</tr>
<tr>
<td>MR</td>
<td>Ln</td>
<td>1.29 (1.08–1.59)</td>
<td>A</td>
</tr>
<tr>
<td>MR</td>
<td>Lp</td>
<td>1.788 (1.38–5.18)</td>
<td>A</td>
</tr>
<tr>
<td>MR</td>
<td>Ms</td>
<td>2.73 (2.26–4.42)</td>
<td>AB</td>
</tr>
<tr>
<td>WL</td>
<td>Ac</td>
<td>1.65 (1.03–3.29)</td>
<td>A</td>
</tr>
<tr>
<td>WL</td>
<td>Aw</td>
<td>1.20 (0.92–1.57)</td>
<td>A</td>
</tr>
<tr>
<td>WL</td>
<td>Ln</td>
<td>0.77 (0.57–1.38)</td>
<td>A</td>
</tr>
<tr>
<td>WL</td>
<td>Lp</td>
<td>0.77 (0.56–1.24)</td>
<td>A</td>
</tr>
<tr>
<td>WL</td>
<td>Ms</td>
<td>1.43 (1.22–1.89)</td>
<td>A</td>
</tr>
<tr>
<td>YG</td>
<td>Ac</td>
<td>1.29 (1.17–1.45)</td>
<td>A</td>
</tr>
<tr>
<td>YG</td>
<td>Aw</td>
<td>1.34 (1.12–1.88)</td>
<td>A</td>
</tr>
<tr>
<td>YG</td>
<td>Ln</td>
<td>1.05 (0.89–1.21)</td>
<td>A</td>
</tr>
<tr>
<td>YG</td>
<td>Lp</td>
<td>1.07 (0.94–1.23)</td>
<td>A</td>
</tr>
<tr>
<td>YG</td>
<td>Ms</td>
<td>2.45 (2.12–3.32)</td>
<td>B</td>
</tr>
</tbody>
</table>

acted before larval performance in restricting gene flow (see [6, 11] for examples of the complexities of estimating components of reproductive isolation associated with a suite of traits). We stress that these are hypotheses that bear further investigation, as we know that larval performance is complex, being affected not only by variation in host quality (i.e., the availability of flowers [19]) but also by the presence of mutualistic ants and natural enemies [33].

5. Conclusion
We conducted a performance experiment for seven populations from three species within the *Lycaenides* species complex, *L. idas*, *L. melissa*, and the hybrid species, on five different plants. Our primary results include large plant effects, with *L. idas* hosts being generally superior for larval development and the exotic host of *L. melissa* being extremely poor, both
for *L. melissa* and the other taxa. In general, there is little evidence of local adaptation in these performance data. This conclusion is perhaps consistent with the fact that these butterfly taxa are associated with multiple hosts throughout their geographic ranges. Thus gene flow could limit local adaptation to any particular plant species. As a consequence, variation in larval performance across multiple hosts is unlikely to be the dominant mechanism of reproductive isolation between populations and taxa.

Our results (including some evidence for local adaptation among *L. melissa* populations for their native host, *A. canadensis*) together with previously published data [16, 17, 19, 22, 23] were integrated to build a hypothetical model relating ecology to reproductive isolation and diversification in *Lycaenidae*. The model presented in Figure 4 describes a system that is well poised for a test of the “multifarious selection” hypothesis [15]. One hypothesis that can be generated from Figure 4 is that there might not be one single trait that could act as a barrier to gene flow between all three taxa, and most traits might only act to reduce gene flow asymmetrically. For example, egg adhesion could affect gene flow from both *L. idas* and *L. melissa* into the hybrid species, but would not necessarily be effective in the opposite directions (from the hybrid species into *L. idas* and *L. melissa*). More generally in the context of ecological speciation, a greater number of traits might increase the possibility that a hybrid “falls between” the niches represented by the two adaptive peaks occupied by the species or incipient species [34, 35]. In a relatively simple example involving two traits, hybrids between populations of *Mitoura* butterflies associated with different host plant species inherit a maladaptive mismatch of traits: hybrid individuals have higher performance on one of the parental hosts, but express an oviposition preference for the other host [36].

However, the importance of multiple traits for ecological speciation in *Lycaenidae* must wait on estimates of historical and contemporary gene flow between pairs of populations and analyses of those estimates in light of variation in ecological and behavioral traits [37]. The inclusion of such comparative data, particularly for a larger suite of populations, would perhaps reveal the influence of a single trait for explaining a majority of the variation in reproductive isolation. It is also possible that a key trait for reproductive isolation remains unstudied in this system. For future studies in this group, it will also be important to sample populations widely throughout the geographic ranges of the focal butterfly species (Figure 1), as dynamics of local adaptation and diversification can be affected by geographic context, particularly proximity to the edge of a range and potentially marginal habitats. Beyond the details of ecological diversification in *Lycaenidae*, our results should generally stress the importance of delving deeper than the traditional “preference-performance relationship” when investigating ecological speciation in herbivorous insects.

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**References**


Divergent Selection and Then What Not: The Conundrum of Missing Reproductive Isolation in Misty Lake and Stream Stickleback

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In ecological speciation, reproductive isolation evolves as a consequence of adaptation to different selective environments. A frequent contributor to this process is the evolution of positive assortative mate choice between ecotypes. We tested this expectation for lake and inlet stream threespine stickleback (Gasterosteus aculeatus) from the Misty system (Vancouver Island, Canada), which show strong genetically based adaptive divergence and little genetic exchange in nature. This, and work on other stickleback systems, led us to expect positive assortative mating. Yet, our standard “no-choice” laboratory experiment on common-garden fish revealed no evidence for this—despite divergence in traits typically mediating assortative mating in stickleback. These results remind us that divergent natural selection may not inevitably lead to the evolution of positive assortative mate choice. The apparent lack of strong and symmetric reproductive barriers in this system presents a conundrum: why are such barriers not evident despite strong adaptive divergence and low gene flow in nature?

1. Introduction

Ecological speciation occurs when divergent selection causes adaptive divergence that then causes the evolution of reproductive isolation [1]. This process now has considerable empirical support across a wide range of taxa [1–3]. Some of the strongest support comes from laboratory studies showing that mating is more frequent between individuals from similar environments than between individuals from different environments [4–10]. However, some recent work suggests that positive assortative mate choice (henceforth PAMC) does not always evolve in response to divergent selection [11–15]. These results suggest the importance of other modifying factors that influence the evolution of PAMC—even when divergent selection and adaptive divergence are present [16, 17]. Insight into these modifying factors can be gained from empirical systems within which the strength of PAMC varies among population pairs (e.g., [18, 19]).

Threespine stickleback (Gasterosteus aculeatus Linnaeus) are a useful system for investigating ecological speciation because they show dramatic adaptive divergence between populations inhabiting different environments, hereafter referred to as “ecotypes” [20–22]. This adaptive divergence appears linked to PAMC in experimental studies on a number of ecotype pairs: benthic versus limnetic [5, 8, 9, 23–25], fresh water versus anadromous [7, 26], and mud versus lava [27]. By contrast, PAMC was not found in recent comparisons of fresh water versus anadromous [28] and lake versus stream stickleback [29]. Our study will further explore the lake/stream situation.

Several characteristics make lake/stream stickleback suitable for the study of ecological speciation. First, lake/stream population pairs have evolved independently in many different watersheds following postglacial colonization by similar marine ancestors [30–33]. Second, lake/stream stickleback often show genetically based adaptive divergence in phenotypic traits related to foraging, swimming, and...
predator avoidance [31, 32, 34–40]. Third, lake/stream stickleback are often parapatric, and different population pairs show different levels of gene flow. In particular, the lake/stream pairs that show the greatest adaptive divergence are also those that show the lowest gene flow at neutral markers [31, 33, 38, 39, 41]. This last pattern is often used to infer progress toward ecological speciation, but it has two limitations: (1) cause and effect are hard to disentangle [42] and (2) neutral markers are often insensitive indicators of progress toward ecological speciation [43–45]. It is therefore important to also test for specific reproductive barriers thought to have an ecological basis [16].

Our investigation of reproductive barriers in lake/stream stickleback focuses on populations found in the Misty watershed, Vancouver Island, Canada. The Lake and Inlet stream populations in this watershed show strong genetically based adaptive divergence and very low gene flow in neutral markers [37, 38, 40, 46, 47]. They are clearly under strong divergent selection and so might be expected by the theory of ecological speciation to exhibit PAMC. Yet our first test for this possibility did not find any evidence for this [29]. However, the experimental design used in [29] (i.e., pairs of directly interacting males between which females could choose) was quite different from the standard “no-choice” designs that have so frequently revealed PAMC in stickleback (Table S1 of the Supplementary material available online at doi:10.1155/2011/11902438). As experimental setup and environmental conditions may influence the outcome of mate choice studies in various ways (e.g., [48–50]), it is important to repeat these tests in multiple different conditions. We therefore here revisit the question by analyzing mate choice experiments done with the “no-choice” design. This allows us to (1) see if the apparent lack of PAMC between Misty Lake and Inlet stickleback is robust to experimental methods, and (2) make more direct comparisons to previous work on other stickleback systems. In addition, whereas the study by Raeymaekers et al. [29] investigated mate choice of lake and inlet females towards lake, inlet, and hybrid males, we include a “control” comparison of Misty Lake fish versus Misty Outlet stream fish. These two populations show very low adaptive divergence and very high gene flow in nature [32, 38, 39, 41, 46, 47, 51]. This comparison can therefore serve as a low divergence lake/stream “control” in which we have no reason to expect PAMC—in contrast to the high-divergence comparison of Misty Lake versus Misty Inlet.

Some other features of our study design are important to note. First, our experimental fish were reared for their entire lives in a common garden environment, as opposed to being collected from the wild. This was done to reduce the chance that observed mate choice patterns result from environmental (nongenetic) effects, such as imprinting, maternal effects, prior experience, and/or other forms of plasticity (e.g., [48–50]). Second, we presented individual males of each type (Lake, Inlet, and Outlet) to females of all three types, which should increase inferential ability by controlling for variation among individual males. Our main prediction is that if PAMC contributes to reproductive isolation in the Misty watershed, we should see PAMC between the strongly divergent Lake and Inlet fish, but not between the phenotypically and genetically similar Lake and Outlet fish: Inlet females should prefer Inlet males and choose against both Lake and Outlet males, whereas both Lake and Outlet females should prefer Lake and Outlet males and choose against Inlet males.

2. Materials and Methods

2.1. Collections and Rearing. In June, parental fish were collected from the Misty system at one Lake site (site 1), one Inlet site (site 4, ca. 2.6 km from the lake), and one Outlet site (site 4, ca. 0.85 km from the lake) (for a map, see [47]). Standard artificial crossing methods [52] were used to generate eight full sibling families for the Lake population, seven for the Outlet, and four for the Inlet. The smaller number of Inlet families was due to limited availability of mature females during the collection period. Fertilized eggs were transferred to our laboratory at McGill University, Montreal, where rearing proceeded in family-specific groups in 20–100 L tanks (aquaria), with a given family split into multiple tanks randomized across four climate-controlled chambers. Fish were maintained at roughly equal densities (25 fish per 100 L), and families were never mixed. Males and females could not be visually discriminated as juveniles, and so were raised together. As fry, the fish were initially fed live brine shrimp nauplii (Artemia sp.) and then later switched to a mixed diet of live brine shrimp nauplii, frozen blood worms (Chironomid sp.), and frozen brine shrimp. During the conditioning period (see below), the fish were fed a mixture of live brine shrimp nauplii and live blackworms (Lumbriculus sp.).

Through April of the following year, the fish were reared under constant “summer” conditions (16:8 L:D photoperiod, 17°C), after which they were switched to “winter” conditions (8L:16D, 12°C). The different chambers were then switched back to “summer” conditions at various times from September through October to ensure the availability of fish in breeding condition over an extended period of time. No nesting material was provided in the rearing tanks, thus preventing breeding activities prior to the experimental trials described below. Fish were used in the experiment once they reached sexual maturity, as indicated by nuptial color in males and distended abdomens in females. This laboratory cohort of fish has been previously analyzed for morphological traits [46], male courtship [51], and swimming performance [40] and represent the parental generation for the fish used in the experiments of [29, 53].

2.2. The Experiment. Eighteen experimental tanks (102 L; length = 92 cm × width = 32 cm × depth = 39 cm), each filled with water to a depth of 20 cm, were set up in a shaded green house at McGill University. The bottom of each tank was covered with dark gravel, and one end was additionally covered with a patch of sand (length = 15 cm × width = 32 cm × depth = 2.5 cm). Dead plant matter collected from Misty Lake, plus a small amount of green algae and grass clippings, was provided as nest building material because pilot studies with wild-caught fish showed that this material was readily used for nest building by all ecotypes. All sides
of the tanks were covered with brown packing paper to minimize disturbance.

The experiment was run under “summer” conditions (16L : 8D, 18°C). A single male was placed into each tank and was then stimulated to build a nest by periodic exposures (15 minutes once or twice per day) to a gravid female. These “stimulus” females were chosen haphazardly from the rearing tanks, were presented in a clear glass jar with a mesh lid, and were not used in the experimental trials described below. A male was assumed to be ready for a trial when he showed nuptial coloration and guarded a nest. Males that did not build nests within a week were not used in the experiment. As all males faced multiple randomly chosen females during the stimulation period, it is unlikely that the identity of these females would have biased male behavior during the mating experiment. After a male was removed from a tank, the tank was cleaned, all materials were replaced, and a new male was added.

Prior to the experimental trials, the fish had been reared under a combination of natural and full-spectrum fluorescent light (Vita-Lite 40 W, Duro-Test, Canada). The Misty system, however, is highly tannic [37] and is dominated by red light (confirmed by spectroradiometer measurements; N. P. Millar, unpubl. data). We mimicked these lighting conditions during the experimental trials by filtering full-spectrum fluorescent light through a filter (rust, code 777, Lee Filters, England) placed above each tank.

An experimental trial was initiated by allowing a gravid female to swim freely from a clear plastic box into a male’s tank. Subsequent male-female interactions were then videotaped with a digital camcorder (Canon ZR90; Canon, see [51] for details). Video recording stopped after 45 minutes or after the female entered the nest, whichever came first. After each mating trial, ripeness of the eggs of each female was confirmed by gentle pressure on her belly (ripe eggs require only gentle pressure to extrude). Any trial with a nonripe female was disregarded, and the male was tested again with another female of the same ecotype after a lag time of at least three hours.

Each male was sequentially exposed to three females, one of each ecotype, at minimum intervals of three hours. The sequence in which different female types were introduced to a male was varied among males so as to achieve a similar sequence distribution across the male ecotypes. Some deviations from a perfectly balanced design occurred when gravid females of the right type were not available at the right time. Nine males were therefore tested with only two female ecotypes and one male with only one female ecotype. Excluding these males did not influence our conclusions, and so all males are included in the analyses presented below. Each female was used in only a single trial, and males and females from the same family were not combined in any trial.

In order to standardize the conditions experienced by one male across all three female types, we prevented actual spawning during the experimental trials. This was done by using long forceps to gently squeeze the female’s caudal peduncle if she entered the nest, which always precipitated her immediate departure. After the trials for a given male or female were complete, the fish was anesthetized with buffered MS222, measured for standard length, and photographed on a grid-ruled background (Nikon coolpix 5400, Nikon Inc.). A total of 111 trials were conducted on 43 males. Total numbers of trials for the Inlet (I), Outlet (O), and Lake (L) ecotype combinations were II = 12, IL = 13, IO = 14, LI = 12, LL = 14, LO = 13, OL = 12, OL = 11, and OO = 10 (male origin indicated by first letter, female origin by the second letter).

2.3. Response Variables. Our three measures of female behavior were (1) head up (the female inclines her head and body upwards in a roughly 45° angle), (2) nest inspection (the female places her snout in the nest opening), and (3) nest entry (the female enters the nest). These variables represent progressive stages during the mating sequence. Head up indicates female responsiveness, and nest inspection indicates a transition between female responsiveness and female choice. Nest entry indicates female choice and is strongly correlated with spawning (pers. obs.). (Note. Females can skip any of these steps.) Each of these variables has been used in previous work on stickleback (e.g., [25, 52, 54]), and they are here treated as binomial variables (i.e., whether or not a given female showed a given response when interacting with a given male).

To test whether mating is nonrandom with respect to phenotype, we analyzed female mate choice in relation to body size and male courtship. Body size is of particular interest as it has been suggested to be a “magic trait” driving ecologically mediated PAMC in stickleback through a “mate with your own size” rule [7, 8, 24]. Our two body size metrics were relative size difference (male size minus female size, henceforth RSD) and absolute size difference (larger sex minus smaller sex, henceforth ASD)—both indicative of size assortative mating. (We also tested for an effect of absolute male size, but as that had very minimal effects and did not alter any of our conclusions, we do not present the results here.) Our two measures of male behavior were aggression and display toward females. Aggression and display were both composite variables calculated as the sum of the frequencies of individual behaviors that males showed within each of these behavioral types during the first 15 minutes of each trial (for details see [51]). As the aim of the male courtship analyses was to estimate ecotype-specific male behavior, only the first 15 minutes were used to reduce bias from female behaviors on male behaviors. Both aspects of behavior are thought to influence mate choice in stickleback [55–57] and differ among the ecotypes within the Misty system, particularly by Lake (and Outlet) males showing more “aggressive” behaviors than Inlet males do [51].

2.4. Statistical Analyses. All statistical analyses were conducted in SAS 9.2 (SAS Institute, Inc.). Female behaviors were analyzed with generalized linear mixed models (Proc glimmix) using Maximum likelihood models with a Laplace approximation, binomial error structure, and a logit link function [58]. As an immediate test for PAMC, we first ran “ecotype models” (one for each female response variable) that included the fixed effects of male ecotype, female ecotype, and their interaction. In these models, random effects included male identity (nested within male ecotype)
and the intercept, but not family (the data were insufficient to do so for binomial response variables). Also not included in the analyses presented here was the order of presentation of female ecotypes to a given male because this term was never significant. Adjusted post hoc Tukey tests based on least square means were used to assess pairwise differences among different ecotypes when relevant. To strengthen our conclusions, we confirmed our results for nest entry (the core measure of mating isolation) also in two other ways. First, by scoring each female response variable from 0 (none of the responses) to 3 (nest entry alone or together with headup and/or nest inspection) and conducted a generalized linear mixed model with an ordered multinomial response. Second, we treated both lake and outlet fish as the same ecotype (“lake”) and analyzed the ecotype model comparing Inlet versus “Lake”. However, as the results in both of these analyses were similar to our present analyses, these results are not further reported.

We next considered divergence in traits (body size and male behavior) that might influence mating patterns. In our previous paper [51], we analyzed male behavior in more detail, but not its influence on female preferences (which we do here). As a few males included in [51] were not included in our mating trials, we here confirm ecotype specific trait values and their statistical main effects (Table 2 and Table S2). Body size (log-transformed) and male behaviors (square-root-transformed) were analyzed separately. Body size divergence (individual standard length) among ecotypes was analyzed within each sex with mixed model analyses of variance (Proc mixed), where fixed effects were male (or female) ecotype, and family (nested within ecotype) (nesting being possible for the continuous data used here). Male behavioral traits were aggression (frequency of aggressive behaviors during a given mating trial) and display (frequency of display behaviors during a given mating trial). Finally, we ran “covariate models” to test whether mating is nonrandom with respect to male phenotype, and whether female ecotypes have diverged in preferences for body size and male behavior. The two body size metrics used as covariates were RSD and ASD, and the two male behavior variables were rates of aggression and display, as defined above. As for the ecotype models, these covariate models included male identity as a random effect (subject), but did not include family effects or the order of presentation of female ecotypes to males. Two modifications to the data were necessary in these covariate models. First, in analyses of head up and nest entry one outlier had to be removed to achieve convergence. Second, a few missing values for body size ($N = 3$) or behavior ($N = 1$) had to be estimated from the ecotype means. These modifications had no effect on our conclusions.

Covariate model analyses started with full models that included fixed effects of male and female ecotype, male phenotype covariates (behaviors and body size), and all two-way interactions between female ecotype and male phenotype. We then generated reduced models by removing sequentially nonsignificant ($P > 0.1$) terms involving covariates. Because display and aggression were strongly correlated and distinct among the male ecotypes, covariate models including both display and aggression, or male ecotype × female ecotype interactions, showed multicollinearity and reduced model stability. We therefore ran four sets of covariate models, each containing male ecotype, female ecotype, and female ecotype × covariate interactions and two covariates: (a) display and RSD, (b) display and ASD, (c) aggression and RSD, or (d) aggression and ASD, but excluding the male ecotype × female ecotype interaction. Although we do not conduct formal model comparisons [59], but run several models on the same data set, we report AICc scores for the ecotype models and for the final (reduced) covariate models to allow some context to their relative explanatory power while discounting for model complexity [59]. Here those models having clearly lower AICc values were considered to have stronger support [59].

2.5. Literature Survey. To allow more direct comparisons of our results with those from other studies, we also surveyed the published literature for studies that have explicitly tested for PAMC between divergent threespine stickleback. Here we included studies that used comparable experimental methodology (either a choice or no-choice setting) and response variables (nest inspection and nest entry/spawning). The details for this survey are given in Table S1.

3. Results

3.1. Ecotype Models: Testing for PAMC. Neither head up nor nest inspection rates depended on male ecotype, female ecotype, or the male ecotype × female ecotype interaction (Figures 1(a) and 1(b), Table 1(a)). Nest entry was marginally influenced by male ecotype, and significantly by female ecotype, but not by a male ecotype × female ecotype interaction (Figure 1(c), Table 1). Outlet males tended to have higher mating success than either Inlet or Lake males (Figure 1(c)); however, these effects were not significant after Tukey adjustment (all pairwise $P > 0.1$). Inlet females were more likely to mate than either Lake (Tukey $P = 0.044$) or Outlet females (Tukey $P = 0.087$, Figure 1(c)). Thus, genetic differences between ecotypes may exist in their propensity to solicit nest entry (males) or enter nests (females), but these differences did not generate PAMC.

3.2. Trait Divergence. Both for males and females, Lake and Outlet fish were similar in size and larger than Inlet fish (Table 2). In the between-type mating trials, Inlet fish therefore tended to be smaller than Lake or Outlet fish (combinations IL, LI, IO, and OI in Figure 2). Confirming results from Delcourt et al. [51], a significant male ecotype effect in aggression and display, and a near significant female ecotype effect in display, (Table 2) was present because Lake and Outlet males were more aggressive but displayed less than Inlet males and because Inlet females tended to elicit more displays from males than did Lake or Outlet females (Table 2). Neither RSD nor ASD had a significant effect on male aggressive behavior (both $P > 0.2$, Table S2), whereas ASD had a significant effect on male display behavior: males displayed more towards relatively smaller females ($\beta \pm$ S.E.: $1.925 \pm 0.662$, $P = 0.005$). However, even correcting for
body size, the male × female ecotype interaction was never significant (P > 0.7 in all cases, Table S2).

3.3. Covariate Models: Testing for Female Trait Preference. We here summarize the significant effects of covariates, with details and nonsignificant effects appearing in Tables 1 and 3. Females showed the head up response more frequently towards males that displayed more, and marginally more towards males that were relatively smaller than females (Figure 2, Tables 1 and 3). Females also inspected more frequently nests of males that showed higher rates of display or aggression (Table 3). Moreover, there was a near significant female ecotype × aggression interaction on nest inspection (Table 1): both Lake and Outlet females showed higher nest inspection rates with greater male aggression, whereas there was no such trend for Inlet females (Table 3). For nest entry, females showed greater responses to males with greater display or aggression (Tables 1 and 3). Females also appeared to more frequently enter nests in combinations where males were relatively larger (Figures 2(b) and 2(c)), but neither of the size metrics had statistically significant effects (Table 1). Significant female ecotype effects arose in the covariate models for head up and nest entry: Outlet females showed stronger head up response (models a, b, and c, Table 1) and Inlet females tended to have higher nest inspection rates than Outlet females (model c, Table 1) (Figures 1(a) and 1(b)). For nest entry, the male and female ecotype effects seen in the ecotype models remained qualitatively similar in

![Figure 1: Frequencies of (a) head up posture, (b) nest inspection, and (c) nest entry for different combinations of male and female ecotype of Misty Inlet, Outlet, and Lake threespine stickleback. The bars present different male ecotypes and are means ± S.E. of binomial frequencies (yes/no).](image-url)
Table 1: Generalized linear mixed model results for (I) ecotype and (II) covariate models testing for effects of male ecotype (meco), female ecotype (feco), and relevant covariates on head up, nest inspection, and nest entry. Mid stands for male identity. Covariates included in the initial full models were (a) display and RSD, (b) display and RSD, (c) aggression and ASD, or (d) aggression and ASD. Ecotype model and final (reduced model) AICc are given to allow inference of relative support of different models, whereby lower (difference > 2) is better [59]. See Table 3 for covariate slopes. Significant effects are highlighted in bold and marginally significant (P < 0.1) in italics.

<table>
<thead>
<tr>
<th>Model type</th>
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<th>AICc</th>
<th>Head up</th>
<th>Nest inspection</th>
<th>Nest entry</th>
</tr>
</thead>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Random</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mid (meco)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
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<td>0.725</td>
<td>38 0.29</td>
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<td>0.00</td>
<td>1.000</td>
<td>62 1.18</td>
</tr>
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<td>62</td>
<td>0.19</td>
<td>0.943</td>
<td>62 0.19</td>
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<td></td>
</tr>
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<td>(a) Display and RSD</td>
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</tr>
<tr>
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<td></td>
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</tr>
<tr>
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<td>0.455</td>
<td>3.188</td>
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<tr>
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<td>1.18</td>
<td>0.318</td>
<td>38 2.59</td>
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<td>65 8.50</td>
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<tr>
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<td>3.414</td>
<td>0.455</td>
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<td>38</td>
<td>1.18</td>
<td>0.318</td>
<td>38 2.59</td>
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<td>65 1.31</td>
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<td>(c) Aggression and RSD</td>
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<tr>
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<tr>
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<td>4.93</td>
<td><strong>0.010</strong></td>
<td>63 4.01</td>
</tr>
<tr>
<td>Aggression</td>
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<td>9.47</td>
<td><strong>0.003</strong></td>
<td>63 6.50</td>
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<td>(d) Aggression and RSD</td>
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3.4. Summary of Main Findings. Taken together our analyses on female mating preferences, and those on male courtship behavior (see also [51]), yielded five basic conclusions. First, there is no evidence for PAMC among the ecotypes. Second, Lake and Outlet males show more aggressive courtship [51] than Inlet males, but males of all three ecotypes display more towards relatively smaller (Inlet) females. Third, female mate choice was nonrandom with respect to male phenotype: some covariates were highly significant and the covariate models clearly had more support (much lower AICc scores) than did the ecotype models. Fourth, females tended to differ in trait preferences: Lake and Outlet females tended to prefer more aggressive males (nest inspection), but this was not
seen for Inlet females. Fifth, there was no evidence for size assortative mate choice: female responses were only weakly affected by gender differences in body size (effect of RSD on head up).

The survey of other PAMC tests in stickleback (Figure 3 and Table S1) revealed that the mating frequencies in our study fell within the range of those in other studies, though being on the lower end for nest inspection. Moreover, in contrast to most other studies, nest entry rates were somewhat higher for different ecotype matings than same ecotype matings in our study.

4. Discussion

We found no evidence of positive assortative mate choice (PAMC) between lake and stream stickleback from the
Table 3: Ecotype main effect LS means ± S.E. and covariate slopes ± S.E. for head up, nest inspection, and nest entry in the Misty Lake (L), Inlet (I), and Outlet (O) three spine stickleback. The values are from final (reduced) covariate models in Table 1. For covariate effects, common slopes are given when there was no significant female ecotype × male trait interaction, whereas female ecotype specific slopes are given when these interactions were at least nearly significant. Models refer to models with (a) display and RSD, (b) display and ASD, (c) aggression and RSD, or (d) aggression and ASD as covariates in initial full model, with results of Tukey adjusted pairwise comparisons given below.

<table>
<thead>
<tr>
<th>Model</th>
<th>Female response</th>
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<th>Nest entry</th>
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<td>(a) Display and RSD</td>
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<tr>
<td>Ecotype main effects</td>
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<tr>
<td>Female ecotype</td>
<td>Inlet</td>
<td>2.556 ± 1.317</td>
<td>−0.926 ± 0.458</td>
</tr>
<tr>
<td></td>
<td>Outlet</td>
<td>4.451 ± 1.701</td>
<td>−1.905 ± 0.591</td>
</tr>
<tr>
<td></td>
<td>Lake</td>
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</tr>
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<td></td>
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<td>ns</td>
</tr>
<tr>
<td></td>
<td>I vs. O</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>L vs. O</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Male ecotype</td>
<td>Inlet</td>
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<td>−2.388 ± 0.745</td>
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<tr>
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<tr>
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<td>I vs. O</td>
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<tr>
<td></td>
<td>L vs. O</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Slopes</td>
<td>Display</td>
<td>1.998 ± 0.877*</td>
<td>0.931 ± 0.319**</td>
</tr>
<tr>
<td></td>
<td>RSD</td>
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<tr>
<td>Ecotype main effects</td>
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<td>Final model same as in a</td>
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<tr>
<td></td>
<td>I vs. O</td>
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<td></td>
<td>L vs. O</td>
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<tr>
<td></td>
<td>I vs. O</td>
<td>*</td>
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</tr>
<tr>
<td></td>
<td>L vs. O</td>
<td>**</td>
<td>ns</td>
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<tr>
<td>Slopes</td>
<td>Display</td>
<td>1.373 ± 0.486**</td>
<td>4.977 ± 1.711**</td>
</tr>
<tr>
<td></td>
<td>RSD</td>
<td>—</td>
<td>1.135 ± 0.352**</td>
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<td>(c) Aggression and RSD</td>
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Final models same as in c

* P: * < 0.1, * < 0.05, ** < 0.01, *** < 0.001.
Misty system. This was not surprising for Lake versus Outlet fish given their phenotypic and genetic similarity, and so we do not further discuss that result. By contrast, the lack of PAMC between Lake and Inlet fish (which is also found for a different lab cohort and with a different experimental design, [29]) is surprising. That is, PAMC might be expected given (1) strong adaptive divergence in traits typically associated with mate choice in stickleback, in particular, body size and male behavior, (2) very low gene flow between these populations in nature, and (3) previous demonstrations of PAMC between other stickleback ecotypes (see Introduction). In the following sections, we first discuss factors that might influence this negative outcome, paying particular attention to experimental design and biological factors suggested to be important in theory and in stickleback and compare our studies with previous studies testing for PAMC in stickleback (Figure 3; Table S1). We then discuss how our results might relate to pressing questions in the study of ecological speciation.

4.1. Experimental Design. Perhaps the different outcome of our study versus previous work on other stickleback systems (Table S1) stems from differences in experimental design. First, our experiment might have lacked the statistical power necessary to detect PAMC. We do not think this is the case for three complementary reasons: (1) our statistical control for individual male variation should be a powerful design for detecting ecotype effects, (2) our sample sizes were similar to a number of previous studies that did detect PAMC (Table S1), and (3) the range of observed mating frequencies in our study fell within those seen in other studies (Figure 3). Moreover, although mate choice is clearly not random across the combinations, neither alternative ways of analyzing (see statistical analyses section) nor visual inspection of the data (Figure 1 and Figure S1) suggest any hint of positive assortative mating.

Second, laboratory mate choice experiments are always conducted under artificial conditions and thus exclude genotype-by-environment interactions that might be important in nature (e.g., [49, 60]). Regardless, our experimental tanks and our no-choice design were very similar to those used in several previous studies that did detect PAMC in stickleback (Table S1), and we also did not detect PAMC using a different experimental design [29]. Our fish originated from multiple full-sib families, but we could not account statistically for family effects in our mate choice analyses (the data was insufficient to do so), and we can therefore not fully discount that family effects would have influenced our analyses. However, we think that any such effects would likely have been small compared to the ecotype differences, as supported also by the observation that divergence in traits typically mediating mate choice (behavior, body size, and body shape) remained strong even after accounting for family effects [46, 51]. We therefore suggest that differences in experimental venue and design are unlikely to have caused the difference between our results and those documented in other stickleback systems.

Third, the present study and that of Raeymaekers et al. [29] both used common-garden fish, whereas nearly all previous stickleback studies have used wild-caught fish (Table S1, Figure 3, with an exception of [48] and [52]). When wild-caught fish are used, observed mating patterns could reflect environmental (nongenetic) effects, such as imprinting, prior experience, or some other form of phenotypic plasticity (e.g., [48–50]). Studies testing for PAMC would benefit from employing both common-garden and wild-caught individuals because this allows complementary inferences: common-garden individuals inform whether or not genetic divergence in mate choice has occurred, whereas wild-caught individuals might be more reflective of actual mating patterns in nature. In addition, complementary studies on PAMC in the wild would allow strong inferences about

![Figure 3: The relationship between same ecotype and different ecotype rates of (a) nest inspection and (b) nest entry or spawning in previously published threespine stickleback studies (open or grey circles) and current study (solid symbols). Open circles: wild-caught fish, grey circles: lab reared fish. Solid triangles: LO or OL combinations, which are treated as same ecotype due to phenotypic and genetic similarity. The line represents the 1:1 line of same and different ecotype matings. The details of the included studies are given in Table S1.](image-url)
the true extent of PAMC. Combined experiments of this sort could make important contributions to the current debates about the importance of plasticity in promoting versus constraining ecological speciation and adaptive radiation [61–65].

4.2. The Role of Trait Divergence. In the context of ecological speciation, PAMC is expected to arise as a result of adaptive divergence in traits [66]. We consider two important factors that might contribute to this association. First, it has been argued that substantial progress toward ecological speciation, whether through PAMC or other reproductive barriers, requires either strong divergent selection on one ecological/trait dimension or modest-to-strong divergent selection along multiple ecological/trait dimensions [17]. Second, the evolution of mating isolation during ecological speciation is more likely when the traits undergoing adaptive divergence and the traits influencing mate choice are genetically associated [67, 68], so-called “magic traits” [69, 70].

With respect to the strength and dimensionality of divergent selection, Misty Lake-Inlet stickleback should be well endowed: they differ dramatically in a number of traits thought to be under divergent selection. These include body size, morphological traits related to predator defense (e.g., spine length and male color), foraging (e.g., mouth shape and gill raker number and length), swimming performance (e.g., sustained and burst swimming and maneuverability), and male reproductive behavior (e.g., nest construction, aggression, and courtship) [29, 37, 38, 40, 46, 51, 53]. Moreover, common-garden fish maintain divergence in these traits (see above citations), making them relevant in the present experiment. Given all of these trait differences, it seems unlikely that the apparent lack of PAMC is the result of insufficient divergent selection in either strength or dimensionality. Even strong and multifarious selection will not inevitably lead to PAMC. It is theoretically possible that lack of sufficient time for the evolution of mating isolation could contribute to the lack of PAMC. However, we do not think this is the case here, as divergence times based on neutral markers in the Misty system are similar to those in other lake/stream systems that show similar patterns of trait divergence [30, 32, 38]. Likewise, the time frame for colonization is similar in several types of stickleback ecotype pairs, which do show PAMC (i.e., after the last ice age, [21]).

With respect to magic traits, the most widely discussed possibility for stickleback is body size (Table S1 and references therein). As noted above, this trait differs markedly between Misty Lake and Inlet fish in the wild and in the lab. However, the suggested “mate with your own size” rule for stickleback (e.g., [7, 24]) was clearly absent in our experiment (Figure 2): males of all types tended to display more towards smaller (Inlet) females [51] and nest entry was more frequent when males were larger than females (Figure 2(b)). Possible explanations for these patterns are that (1) larger females might be avoided because they more frequently cannibalize the eggs in male nests [71], (2) larger males are of higher quality within both habitats (e.g., [72, 73]), and (3) females have retained ancestral preferences for large males or show a “supernormality” response (i.e., exaggerated version of a stimulus that causes a strong response) (e.g., [74, 75]).

Another possible magic trait would be male reproductive behavior, which is divergent between the ecotypes and influences mate choice. In particular, Lake and Outlet females tended to more frequently inspect the nests of more aggressive males, and Lake and Outlet males were the most aggressive. However, even this correlated divergence of male traits and female preferences was not sufficient to generate PAMC. It is of course possible that the role of male trait divergence and correlated female preference may only be apparent in more natural conditions. For example, if a more aggressive male courtship reflects his ability to defend his territory and offspring (e.g., [54, 71]), females might favor more aggressive males in the lake environments (i.e., high population densities and likely higher predation and egg raiding risk) in nature. Or maybe a distinction is needed between potential magic traits that are “trivial” versus “important,” depending on just how much they contribute to mating isolation [70]. It is also possible that interactions between divergent natural and divergent sexual selection acting on traits, or selection acting in different directions in males and females, (e.g., [11, 15, 25]) may have undermined the evolution of PAMC.

4.3. Relevance for Key Questions in Ecological Speciation. Assuming that PAMC really is absent between Misty Lake and Inlet stickleback, as our work suggests, what might this, together with the frequent evidence for PAMC in other stickleback systems, tell us about ecological speciation? One question relates to the geographical context: close physical proximity between groups under divergent selection (i.e., sympathy or broad parapatry) can both constrain and promote the evolution of assortative mating. The potential constraint occurs when high gene flow hinders the development of a genetic association between adaptive traits and preferences for those traits (recombination: [43, 76]). The potential promoter comes in the form of direct [77] or indirect (e.g., “reinforcement”; [78]) selection to avoid mating with the opposite type.

Previous work on stickleback suggests both possibilities. On the constraint side, high gene flow has clearly constrained adaptive divergence and progress toward ecological speciation in some lake/stream pairs [31–33, 41]. On the promoting side, sympathy seems to enhance PAMC: PAMC is typically present between benthic and limnetic stickleback (Table S1), but it is higher when they come from the same lake [5, 71] than from different lakes [5, 9]. In our case, the Lake and Inlet stickleback populations are parapatric, but our particular inlet sampling site was relatively far from the lake where gene flow from the lake population is probably very low [47]. This suggests that PAMC may be less likely to evolve in this essentially allopatric situation. This hypothesis could be tested in lake/stream systems by conducting experiments with stream stickleback both from very near and far to the lake.

Another question relates to the number and type of reproductive barriers that drive ecological speciation [79, 80]. That is, ecological speciation might sometimes be driven
by only a single barrier that renders all other barriers unimportant. Such a barrier would have to be strong and symmetric and would most likely occur early in the life cycle. Putative examples of such barriers include habitat choice (e.g., [81, 82]), divergent reproductive timing (e.g., [83, 84]), strong viability selection against migrants (e.g., [79, 85]), or postzygotic incompatibilities (e.g., [86]). Alternatively, ecological speciation could be the result of the joint evolution of many reproductive barriers that are strong and symmetric only in aggregate. Along this vein, a number of studies have documented many reproductive barriers between diverging taxa, such as in Mimulus monkeyflowers [87] and Timema walking sticks [18]. However, it remains unclear to what extent these multiple barriers contributed to reproductive isolation during the course of speciation, as opposed to accumulating incidentally or after the fact.

Work on the benthic/limnetic stickleback system suggests that multiple reproductive barriers help to maintain reproductive isolation ([21, 22]). However, the relative importance of each of these barriers during the process of divergence is uncertain. For the lake/stream stickleback system, the problem is the opposite: no strong barriers have been found despite limited gene flow in nature. Instead, the various potential barriers investigated so far seem asymmetric and certainly incomplete, including limited dispersal ability (distance and beaver dams), habitat choice [38], selection against migrants ([38]; K. Räsänen and A.P. Hendry, unpubl. data), temporal isolation (J.-S. Moore and K. Räsänen, unpubl. data), and sexual selection against hybrids [29]. No clear evidence exists either for the role of postzygotic incompatibilities, as fertilization success and survival to hatching are high in hybrids ([37], Räsänen, K., pers. obs.). Overall reproductive isolation might therefore reflect a combination of many different partial reproductive barriers.

Lake/stream stickleback thus present us with a conundrum that could ultimately be very informative of the factors that influence progress toward ecological speciation. Clearly more studies, replicated over multiple systems, are needed on PAMC and other potential reproductive barriers. Of particular utility is the availability of many independently derived parapatric population pairs that show various levels of geographical separation, adaptive divergence, and gene flow [31–33]. Lake/stream stickleback therefore provide an opportunity to quantify the relative importance and symmetry of multiple potential reproductive barriers during the early stages of divergence [79, 80] as well as interactions between ecology and the geographical context in the development of these barriers (e.g., [15, 19, 88]). This, and studies on other taxa with similar levels of variation and replication, together with tests of PAMC in wild and common garden populations, should take us a step closer to understanding the key features of ecological speciation.

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References


Research Article
How Facilitation May Interfere with Ecological Speciation

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Compared to the vast literature linking competitive interactions and speciation, attempts to understand the role of facilitation for evolutionary diversification remain scarce. Yet, community ecologists now recognize the importance of positive interactions within plant communities. Here, we examine how facilitation may interfere with the mechanisms of ecological speciation. We argue that facilitation is likely to (1) maintain gene flow among incipient species by enabling cooccurrence of adapted and maladapted forms in marginal habitats and (2) increase fitness of introgressed forms and limit reinforcement in secondary contact zones. Alternatively, we present how facilitation may favour colonization of marginal habitats and thus enhance local adaptation and ecological speciation. Therefore, facilitation may impede or pave the way for ecological speciation. Using a simple spatially and genetically explicit modelling framework, we illustrate and propose some first testable ideas about how, when, and where facilitation may act as a cohesive force for ecological speciation. These hypotheses and the modelling framework proposed should stimulate further empirical and theoretical research examining the role of both competitive and positive interactions in the formation of incipient species.

1. Introduction

Over the last 20 years, there has been increasing recognition that besides competition, facilitation, that is, the amelioration of biotic and/or abiotic conditions by neighboring organisms, is a key driver controlling assembly and dynamics of communities ([1], see [2] for recent reviews). The impact of facilitation in a community ecology context has been now widely accepted, but its evolutionary consequences have been rarely explored [2, 3]. There also has been an increasing awareness that positive and negative interactions usually cooccur in nature, and recent works have turned to examine conditions under which the outcome of biotic interactions is positive, negative, or neutral [4–6]. Yet, while there is a long-standing literature linking competitive interactions and speciation [7–10], the role of facilitation for evolutionary diversification has been largely overlooked. Very recently, a few empirical studies have attempted to bridge this gap [11–13].

The theoretical study of positive interactions in an evolutionary context has an old history (e.g., [14]), but these models only consider mutualism, a two-species interaction that benefits both partners [15]. However, mutualism is characterized by balanced reciprocal benefits for species involved in a one-to-one relationship, while facilitation encompasses a larger spectrum of positive interactions between organisms [2, 16]. Namely, it commonly includes one-to-many interactions where one organism ameliorates habitat conditions for several beneficiary organisms and many-to-one interactions where an organism benefits from diffuse facilitation by neighbors. For example, facilitation between plants occurs when neighboring vegetation or a nurse plant (more
generally called a benefactor) ameliorates environmental conditions directly, through the provision of additional resources or shelter from extreme conditions (e.g., high or low temperatures), or indirectly through protection from herbivores or via attraction of pollinators [16]. Since facilitation generates local variation in environmental conditions, it should also affect the manner in which organisms respond and adapt to their environment. However, it is important to realize that facilitation differs intrinsically from microhabitat amelioration due to abiotic components of the system (e.g., rock, ponds, or nutrient patches) because the source of environmental heterogeneity is a living organism. For instance, the distribution of favourable microhabitats may change at the time scale of ecological responses because of the population dynamics of benefactor species [17]. Additionally, the net effect of benefactors can shift from facilitation to competition due to interannual resource dynamics (e.g., change in rainfall pulse [18]).

The growing interest for eco-evolutionary dynamics, that is, the interplay between ecological and evolutionary processes [19–22], demonstrates the need to study both the evolutionary consequences of ecological processes and the effect of evolutionary processes on ecological responses. In a recent study, Michalek et al. [17] provided one specific aspect of the problem from the benefactor’s point of view. Namely, they could show that ecotypic variation in benefactor traits may modify the manner in which they affect understory species. Vice versa, the understory also affected fitness of the benefactor. Here, we tackle this problem by looking at the other side of this coin and from a more general perspective, that is, the evolutionary consequences of facilitation on diversification of beneficiaries.

We aim at complementing the vast literature on competitive effects on evolutionary diversification by a conceptual model addressing the role of facilitation on distribution range, local adaptation, and genetic structure of beneficiary species. In particular, we discuss the likely effect of facilitation on microevolutionary processes and ultimately on ecological speciation. Ecological speciation can be defined as a particular type of speciation, where reproductive isolation and evolutionary processes are the most tantalizing [3, 17, 26].

We do not present an exhaustive list of all potential evolutionary consequences of facilitation but instead propose an alternative to traditional views on the role of biotic interactions for evolutionary processes. Our overarching hypothesis is that, contrary to competition, facilitation may impede ecological speciation by maintaining gene flow between incipient species and preventing niche partitioning. Alternatively, we also discuss under which more specific circumstances facilitation could act as a stepping stone to promote ecological speciation. First, we highlight the role of facilitation in the context of niche theory. Secondly, we present how facilitation is likely to interfere with microevolutionary processes and propose a modeling framework to illustrate our arguments (Box 1). Lastly, we call for a new body of empirical and theoretical works to test the presented hypotheses (Box 2). The ideas and hypotheses developed in this paper are biased towards the plant kingdom (as is the facilitation literature in general, e.g., [2, 16]) but should apply equally to other kingdoms. For example, there is now some evidence showing the importance of facilitation for a wide range of organisms such as terrestrial animals [27], marine invertebrates [28], fungi [29], bacteria [3], and viruses [30].

2. Facilitation and Niche Theory, Moving from Species to Genotypes

Facilitation is likely to be as common as competition along environmental gradients [2]. It is generally hypothesized to increase in importance as environmental severity increases ([1], but see [31]) and is likely to enhance species diversity in harsh habitats [31–34]. It is now recognized that facilitation can expand the distribution range of a species at its harsher end, or marginal habitat [35], whereas competition generally reduces it at the favorable end of the environmental gradient [4, 5, 36, 37]. By widening the distribution range of certain species not adapted to stressful conditions, facilitation allows them to persist with subordinate [4, 5, 33, 34, 36] or even dominant status in environments, where without facilitation they would not be able to persist [37]. Therefore, within a particular community, the ability of a species to be facilitated is strongly linked with its own niche [4, 5, 37] in that only species deviated from their niche optima are likely to be facilitated. More specifically, competitive species dominating favorable environments are more affected by facilitation as stress increases than stress-tolerant species adapted to harsher ecological conditions. Conversely, stress-tolerant species are competitively excluded as environmental stress decreases [31] due to a trade-off between competitive ability and stress-tolerance (see [5]).

Here, we apply these concepts developed on a species level to variation among incipient species. Namely, we propose to establish a missing link between facilitation as a force structuring communities and one affecting population structure. Due to the fact that different genotypes within a species may differ in their resource use and stress tolerance [38, 39], they can be characterized by contrasting ecological tolerances and different optima. For example, local adaptation along environmental gradients [40] will lead to ecotype-specific “realized niches.” Note that this concept is commonly incorporated in models of resource competition (e.g., [41, 42]). On the one hand, genotypes exhibiting traits linked to stress tolerance should be selected for, and thus be more common at the margin of the species’ realized niche (harsh conditions). On the other hand, genotypes enabling larger competitive response ability and low stress tolerance should dominate in populations in the center of the species niche, that is, more in favorable environments [43, 44].

Any ecological process that has the potential to significantly impact gene flow in marginal habitats may play a key role in ecological speciation [45, 46]. In that context, dispersal-related processes have been well investigated [43]. Here, we argue that the likelihood that core genotypes can
persists in a species' ecological margin without facilitation is particularly low. In the likely scenario of net dispersal from core (source) to marginal habitats (sinks) [43], facilitation may enhance the establishment of core genotypes in habitats where they otherwise would not be able to persist. This will have crucial consequences for adaptation to marginal habitats, and in turn for species diversification along environmental gradients, which we explain below.

3. Consequence of Facilitation for Gene Flow and Genetic Structure of Beneficiaries

A first mechanism by which facilitation may impact adaptive diversification is in enabling maladapted genotypes/forms to persist and reproduce outside of their niche optima. Figure 1 depicts two habitat types with contrasting environmental conditions (core and marginal), where different genotypes are distributed. Genotypes are supposed to differ by a key trait under selective pressure (e.g., a stress-tolerance-related trait). Local adaptation to marginal habitats is possible if gene flow from core habitats is limited due to pre- or postzygotic barriers ([47], but see [48]). For example, ecological filtering is a prezygotic barrier that will select genotypes best adapted to the local conditions and will reduce viability of the immigrants [49]. Postzygotic barriers may include reduced fitness of hybrids related to stress intolerance or to competitive displacement by more adapted forms [50, 51]. If these mechanisms are sufficiently strong to reduce gene flow between adapted and maladapted forms, trait divergence and ultimately reproductive isolation between populations may be observed (Figure 1(a)). An alternative scenario is depicted in Figure 1(b), where maladapted forms are facilitated by neighboring vegetation, that is, benefactors allow establishment, growth, and reproductive success of
nonoptimal genotypes. In this case, facilitation may cause allele frequencies in the sink (marginal) to be similar to those of the source (core) habitat. The result would be stable coexistence between two genotypes, a core and a marginal genotype, and thus an overall increased genetic diversity and decreased inbreeding but also greater genetic swamping, that is, a decreased potential to adapt to the marginal conditions. Facilitation may thus be a cohesive force that tends to maintain gene flow between these populations and counteracts diversifying selection. To our knowledge, only one experimental field study has tested this hypothesis and supported our conjecture. This study was conducted on the annual grass *Brachypodium distachyon* in the Middle East. *B. distachyon* has a broad distribution range and occurs from Mediterranean (core habitat) to semiarid habitats (marginal habitat). Ecotypic differentiation has been observed in this species, with the semiarid ecotypes being more stress-tolerant [44, 52, 53] and the Mediterranean ecotype having a better ability to cope with competition [44]. When both ecotypes where transplanted to the semiarid environment, both were facilitated by the presence of shrubs but the Mediterranean ecotype could only survive to reproduction underneath the nurse [13]. Facilitation, therefore, enabled a maladapted morph to persist and reproduce in the marginal habitat as described above.

Another scenario would consider the secondary contact of two populations that exhibit trait differentiation. Reinforcement, that is, selection against hybridization, and introgression are two opposing forces that operate during this secondary contact phase, the former enhancing genetic divergence and the latter having the potential to erase it [23]. Secondary contact zones often represent marginal habitats for incipient species (e.g., [54]). Facilitation could favor the cooccurrence of ecotypes according to the mechanism described above and ultimately lead to increased gene flow between ecotypes. Facilitation may also circumvent postzygotic barriers by increasing the fitness of introgressed forms. By increasing the chance of introgression and by preventing reinforcement, we suggest that facilitation could strongly impede further genetic differentiation between ecotypes.

Persistence of core genotypes in marginal habitats increases genetic diversity and thus increases the potential for adapting to changes [46], at least if these occur towards conditions more similar to the core habitat. Higher genetic diversity in marginal habitats compared to core habitats, that is, arid versus Mediterranean environments [55], could support this prediction if this diversity is maintained through time. The trend toward higher genetic variability may be enhanced when the marginal environment varies in time. In that case, the different genotypes inhabiting patches with and without facilitation could have an advantage in different years. Facilitation would then increase the role of temporal variation in supporting stable coexistence of genotypes (e.g., [56]), similar to the storage effect [57] that increases diversity at a species level.

Recent numerical models have been explored under which conditions ecological speciation is likely to occur [51, 58–61]. The hypothesis that facilitation acts as a cohesive force and increases genetic diversity in marginal habitats can be tested with a spatially and genetically explicit modeling framework derived from Kirkpatrick and Barton [58] and Bridle et al. [62] (Box 1). Our simulations confirm that this modeling framework represents a flexible tool to explore the important set of parameters and scenarios that we believe should be investigated (Box 2). We chose here to explore a reduced set of parameters, that is, size of the patch created by the benefactors and the environmental difference between the core and the marginal habitat (Box 1). Our simulations show that, by producing a mosaic of mild conditions in a harsh environment, benefactors increase gene flow between populations from core and marginal habitats [63], that is, lower $F_{ST}$ between populations for both neutral [64] and selected loci (Figures 2(a) and 2(b), resp.). They thus ultimately prevent local adaptation in the harsh environment, as expressed in lower fitness in the marginal environment with facilitation compared to a scenario without facilitation (see Figure 2(c) comparing without benefactors versus smaller patch size benefactors).

### 4. Benefactors and Disruptive Selection

Though facilitation is likely to be a cohesive force, we would also like to touch upon possible conditions under which positive interactions may contribute to, rather than impede, ecological speciation. This is most easily illustrated with particularly stressful environments, where benefactors such as cushion plants in alpine ecosystems or shrubs in water-limited ecosystems [16], represent islands of milder conditions or islands of fertility. At the community scale, this results in a mosaic of microhabitats determined by the distribution and abundance of benefactors. Such systems have provided the most conclusive evidence for facilitation [65], even if the outcome of biotic interactions may strongly depend upon the particular location of the focal species within this mosaic. Arguably, the patchy distribution of biotically engineered favorable microhabitats within a harsher matrix (e.g., shrubs creating islands of fertility in deserts, see Figure 3) may represent a case at hand for disruptive selection between adjacent subpopulations (see [66] for the example of *Bromus erectus* adapting to different *Thymus vulgaris* chemotypes in calcareous grasslands). The benefactors could promote local adaptation in the understory habitats as observed in our simulation when they were forming large patches (Figure 2). Therefore, facilitation could also pave the way to ecological speciation, particularly if directional selection leads to differences in traits that affect the reproductive system, such as flowering time [67]. While this scenario is certainly a possibility, we suggest that it is less likely than the opposite case due to the small spatial scale of environmental heterogeneity usually created by benefactors. Namely, the size of these islands of mild conditions roughly corresponds to the size of the nurse’s crown or to a clump of shrubs and is usually small compared to the dispersal kernel and pollination distance [68]. Furthermore, in extreme habitats the nurses also function as a sink for seeds [69]. As a result, it is unlikely that gene flow will be severely restricted between populations of the understory habitats and those of the harsher matrix. Nonetheless, further empirical and
theoretical studies (Box 2) are needed to test for the overwhelming importance of facilitation as a cohesive, rather than a disruptive, force under these particular environmental conditions.

In the context of colonization of a new habitat, where individuals from the population of the core habitat could not persist away from a benefactor in the first place (Figure 4), benefactors could first promote local adaptation at the niche margin and then in the harsh matrix. Recent studies have pointed out the role of intermediate quality habitats in ecotypic differentiation and in the colonisation of a new niche [70]. In the *Littorina saxatilis* case study, it may be suggested that facilitation played a role in this process given the patchy distribution of mussels and barnacles (in intermediate habitats) associated with the two ecotypes observed in Spain for these species (SU and RB, resp.). However, this hypothesis needs to be properly tested. Thus, on the one hand, positive interactions in intermediate habitats may favor niche broadening and local adaptation. On the other hand, facilitation may contribute to gene flow maintenance between the core and the newly colonized habitats as described in Figure 2. To our opinion, this model system provides excellent opportunities to examine how the spatial and temporal dynamics of positive interactions could interfere with ecological speciation.

5. Facilitation and Niche Conservatism

Linkage between facilitation and macroevolutionary processes has recently arisen from experimental work on semiarid Mexican plant communities [11, 71]. Here, benefactor species were phylogenetically unrelated to beneficiary species; the former belonged to recent Quaternary lineages and were well adapted to the present dry climate, the latter
showed closer affinities with Tertiary lineages and were drought intolerant [11]. It was hypothesized that less adapted “Tertiary” species survive because of facilitation [11]. In this scenario, our model of facilitation acting as a cohesive force is likely to explain niche conservatism of Tertiary species and the fact that they did not become more stress-tolerant or go extinct through evolutionary time. Transposed to the microevolutionary scale, these results suggest that facilitation may cause a genotype’s niche to remain unchanged over time despite environmental changes (Figure 1(c)). Namely, one may imagine that core and marginal habitats are temporally segregated, that is, a population experiences increasingly stressful conditions with time and adaptation to the new conditions is hampered by the persistence of facilitated and maladapted genotypes from more favorable times. Recently, there has been a renewed interest in evaluating niche conservatism across lineages [72], in particular by using species distribution models [73]. To our knowledge, none of these models has properly included the balance between positive and negative interactions along gradients to address the underlying mechanisms and the resulting patterns of niche conservatism.

6. Conclusion

We have argued that facilitation can be viewed as a cohesive force limiting genetic and phenotypic differentiation in marginal habitats. Facilitation may also explain the lack of adaptive diversification over time in certain lineages. On the other hand, under very specific conditions, facilitation could also operate as a stepping stone and pave the way to ecological speciation. This calls for a new body of empirical or theoretical studies addressing the strength of these two possible effects of facilitations on ecological speciation (Box 2). Our focus was on the population scale to provide the linkage with mechanisms of speciation. We thus complement recent opinions about the role of various types of facilitation on macro-evolutionary processes [74] and about the effects of evolutionary processes on facilitative interactions [17]. So far, there is no empirical study that has simultaneously examined genetic population structure of beneficiaries and facilitation for contrasting genotypes/phenotypes in natural conditions. Therefore, there is an urgent need to design novel studies that more closely associate community ecologists and evolutionary biologists to tackle these questions (Box 2).

To conclude, we suggest that plant ecologists should invest more into studying biotic interactions at the population level. First, shifting from species to populations and from population to genotypes in the design of ecological experiments is essential to examine the role of facilitation in ecological speciation [12, 13]. Second, population models should gain more realism by acknowledging the balance between positive and negative interactions along environmental gradients and their underlying ecological mechanisms. This research agenda should help to bridge the gap between community ecology and evolutionary biology. Bruno et al. [36] claimed that “including facilitation in niche theory will challenge some of our most cherished paradigms.” Ecological speciation is another paradigm that is worth revisiting by examining the impact of both negative and positive interactions on the evolutionary history of incipient species. Facilitation is certainly challenging our understanding of the origin of new species.

Boxes.

Box 1: Description of the Modeling Framework. The model of Bridle et al. [62] (initially developed to study adaptation on an environmental gradient) was modified as follows to explore the effect of facilitation on ecological speciation.

The Environment. We used a grid of 8192 × 4096 cells wrapped on a torus. Without facilitation, the left half is the core habitat with an optimum \((U_x \text{ in Bridle et al. [62]})\) of \(\Theta_C\) and the right half is the marginal habitat with optimum of \(\Theta_M\) and reduced carrying capacity \((K_M = K_C/2,\) the carrying capacity if defined on a circle with radius of 50 cells). In the presence of benefactors, the half right-hand side of the grid is fragmented in a checkerboard-like pattern with alternating benefactor and marginal regions. The environments produced by the presence of benefactors have an optimum intermediate between the core and the marginal habitat \((\Theta_F = (\Theta_C + \Theta_M)/2)\) and the same carrying capacity \((K_F = K_C)\) as the core environment.

The Individuals. Individuals are diploid with 128 bi-allelic unlinked loci (64 under selection and 64 neutral). Allele values are either zero or one with a symmetric mutation probability of \(10^{-4}\). The phenotype \((z)\) is the sum of those loci and thus range from zero to 128. The fitness of males and females is determined by a logistic growth and local adaptation

\[
w = \max \left(2 + r_f \left(1 - \frac{N}{K}ight) - s \frac{(\theta - z)^2}{2}, 0\right),
\]
where $w$ if the fitness, $r_f$ is the maximum rate of increase (set to 0.8), $N$ is the local density in a radius of 50 cells, $s$ is the strength of stabilizing selection (set at 0.125, for the two scenarios presented in Figures 2 and 4), and $K$ and $\Theta$ are the carrying capacity and local optimum at the position of the individual. The markers start with no polymorphism (all alleles are zero) and have a mutation rate of $10^{-3}$ that change their value by plus or minus one, so each allele is represented by an integer. We used $F_{ST}$ [75] to infer gene flow among populations using neutral markers and selected loci separately.

**Reproduction.** To find a mate, females scan in a radius of mating distance (MD) set at 150 cells and choose randomly a male with probability proportional to its fitness ($w_{\text{Male}}$). Each couple leaves an average of $w_{\text{Female}}$ offsprings, drawn from a Poisson distribution. Each offspring is placed at a distance from the mother drawn from a normal distribution of mean zero and standard deviation $D$ (set at 100 cells) in a direction randomly selected from a uniform distribution.

**Initial Conditions.** We started each simulation with 500 individuals randomly located in the centre $700 \times 700$ of the core environment, and we follow their evolution over 2000 generations. Three patch sizes were used to simulate the presence of benefactors in the harsh environment, smaller patches (512 cells, isolated benefactors in the landscape), intermediate sizes (1024 cells), and large patch sizes (2048 cells). Each parameter set was replicated ten times (the boxplots in Figures 2 and 4 reflect the ten replicates).

**Example of Two Contrasting Scenarios.** The two sets of simulations that we performed, corresponding to two different scenarios, are presented in Figures 2 and 4. The first scenario presents the case where facilitation inhibits ecological speciation ($\Theta_C = 62$, $\Theta_F = 64$, $\Theta_M = 66$, Figure 2), and the second scenario presents the case where facilitation promotes ecological speciation ($\Theta_C = 61$, $\Theta_F = 64$, $\Theta_M = 67$, Figure 4). Following (1), for the first scenario at carrying capacity in both environments, $w$, the fitness for an individual locally adapted to the core environment ($z = 62$) is equal to 2, but the same individual would have a fitness $w$ equal to 1 in the marginal environment, representing the equivalent of a selection coefficient of 50%. This value is consistent with the average measure of local adaptation recently reported for reciprocal transplantation experiments [76]. In the second
scenario, we set the environmental difference between the core ($\Theta_c = 61$) and the marginal environment ($\Theta_M = 67$) such that individuals locally adapted to the core environment ($z = 61$) could not maintain a viable population in the marginal environment.

**Box 2: Facilitation and Ecological Speciation: Ways Forward**

Stress Gradients as a Model System. For empirically studying the role of facilitation in ecological speciation, one should focus on target species with populations widely distributed along stress gradients, and for which facilitation and trait divergence in marginal habitats have been documented. Common examples are gradients of water availability in semiarid landscapes or gradients of temperature along altitudinal gradients. Population genetic structure and inferences about gene flow could be evaluated first descriptively by using neutral or selected markers and by QTL analyses [77]. The next step would be to investigate adaptive genetic variation between microhabitats with and without benefactor. These should be accompanied by experiments testing whether genotypes from “favorable” habitats rely on facilitation to persist and reproduce in marginal habitats [13]. These questions can be addressed with the same experimental designs that are carried out at the species level, that is, reciprocal transplants or sowing experiments coupled with neighbor manipulations.

Which Traits to Measure? A particular challenge to exploring the effects of facilitation is to identify adaptive genetic variation in traits (1) that are under disruptive selection pressure along the investigated gradient and (2) for which trait expression strongly depends on the balance between stress tolerance and success in the core habitat. Seed size, for example, is a good candidate because it is related to seedling size and tolerance of hazards for young seedlings [78]. As competitive ability and stress tolerances ability are related to the probability of being facilitated [5], traits linked with the resource capture and utilization, such as leaf traits, plant stature traits, and root traits, need to be considered as well (see [79]).

A key trait would be both under strong selective pressure and closely related to mate choice, the so-called “magic traits” [45, 51, 80]. Because the benefactor may modify the length of the favorable season, for instance, phenological traits such as time to flowering or length of the life cycle [44, 81] may fall in this category and are worth examining. Depending on how benefactors affect these key traits, facilitation could potentially either hinder, accelerate or even lead to ecological speciation (see “Section 4”).

**Theory and Simulations.** There are large open avenues for tackling these questions with spatially explicit models of population dynamics, building on a rich set of various modeling frameworks [26, 51, 62, 70, 82–84]. We present here an example (Figures 2 and 4) but such models should be refined to explore the specific feature of facilitation and its implication on adaptive diversification along gradients and other spatiotemporal configurations. Namely, the crucial role of dispersal for adaptation to marginal habitats should be investigated in the presence of facilitation. For example, an important consequence of facilitation may be that it enhances “effective” dispersal from the source to sinks, because establishment probabilities of maladapted genotypes are enhanced. Furthermore, the sensitivity of model outputs to parameters controlling (i) the shape of the competition versus facilitation balance along ecological gradients, that is still widely debated [6, 31], (ii) the spatial distribution and dynamics of nurse plants in marginal habitats, (iii) the complex interactions among benefactors and beneficiaries [17], and (iv) the dispersal ability and life history traits of focal species, has to be investigated. Ultimately, these models should help us sharpen and phrase testable hypotheses about the relative importance of facilitation on adaptation and ecological speciation. Finally, a spatially and genetically explicit model [51, 59–61] could couple both benefactor effect on beneficiaries’ microevolutionary responses (this study) and the feedback effect of beneficiaries on population and evolutionary dynamics of benefactor species (cost of facilitation, [17]). Such an approach would shed light on how the evolutionary consequence of facilitation affects community assembly and dynamics.

**Authors’ Contribution**

P. Liancourt wrote the first draft of the manuscript, X. Thibert-Plante performed the modelling work. All authors made a substantial intellectual contribution to the paper and its revision. P. Choler, N. Gross and X. Thibert-Plante had equal contribution to the paper.

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References


[37] N. Gross, P. Liancourt, P. Choler, K. N. Suding, and S. Lavorel, “Strain and vegetation effects on local limiting resources explain the outcomes of biotic interactions,” *Perspectives in


Research Article

Use of Host-Plant Trait Space by Phytophagous Insects during Host-Associated Differentiation: The Gape-and-Pinch Model

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Ecological speciation via host shifting has contributed to the astonishing diversity of phytophagous insects. The importance for host shifting of trait differences between alternative host plants is well established, but much less is known about trait variation within hosts. I outline a conceptual model, the "gape-and-pinch" (GAP) model, of insect response to host-plant trait variation during host shifting and host-associated differentiation. I offer four hypotheses about insect use of plant trait variation on two alternative hosts, for insects at different stages of host-associated differentiation. Collectively, these hypotheses suggest that insect responses to plant trait variation can favour or oppose critical steps in herbivore diversification. I provide statistical tools for analysing herbivore trait-space use, demonstrate their application for four herbivores of the goldenrods Solidago altissima and S. gigantea, and discuss their broader potential to advance our understanding of diet breadth and ecological speciation in phytophagous insects.

1. Introduction

The insects have long been held up as providing spectacular examples of rapid diversification and high standing diversity (e.g., [1–3]). Among insects, phytophagous clades often undergo dramatic radiations [4], and phytophagous lineages tend to be more diverse than their nonphytophagous sisters [5, 6]. One likely driver of diversification among phytophagous insects is their tendency to specialize on host-plant species or organs [7–10] and to diversify via host or organ shifts followed by host-associated differentiation (HAD), the evolution of new specialist races or species [9, 11–14]. Because many cases of HAD appear to have proceeded in sympatry [15], a great deal of theoretical and empirical work has focused on understanding ways in which adaptation to different host plants can impose disruptive selection on nascent specialist forms and also reduce gene flow (or permit differentiation in the face of gene flow) between those forms [14–16]. Phytophagous insects, along with parasitoids [17], freshwater fishes [18], seed-eating birds [19], and habitat-specialist plants [20] and lizards [21] have therefore been central to the development of ideas about ecological speciation [22].

A common theme among case studies of ecological speciation is the existence of two alternative niches—microhabitats, resources, reproductive strategies, and so forth—that can be exploited by individuals of a single species, with the potential for disruptive selection to operate between the alternative niches. For phytophagous insects, the alternative niches are a pair of host plant species (or organs). One commonly imagines an evolutionary sequence beginning with an insect exploiting only one of the two alternative hosts. Perhaps via host-choice errors, some individuals occasionally attack individuals of the second host, and if fitness penalties for doing so are not too severe, a host shift occurs and the insect begins to exploit both alternative hosts. (Description of these events as “errors” is standard in the plant-insect literature, but of course this usage is teleological shorthand and can conceal interesting biology. For instance, it might be that genotypes with strong enough host preferences to avoid “errors” would also show costly rejection of some suitable hosts; in this case, the occurrence of host-choice “errors” is simply an adaptive compromise. Nonetheless, for simplicity I retain the standard usage here). Disruptive selection can now begin to favour genotypes better adapted to each alternative host. If reproductive isolation arises between nascent forms,
then ecological speciation can proceed, and a single (perhaps polymorphic) generalist is replaced with a pair of host-specialist races or species. Because reproductive isolation is expected to take some time to evolve, if it can evolve at all, different insects exploiting a pair of alternative host plants are expected to fall on a continuum from generalists to nascent, poorly differentiated host forms to distinct host-specialist sister species [13, 23]. There will be analogous continua for ecological speciation across other kinds of alternative niches, for instance, in parasitoids speciating across hosts or fish across depth niches (e.g., [17, 24]).

The process of HAD in phytophagous insects has been widely discussed, both in general [14] and in the context of a few well-studied model systems (e.g., apple maggot fly [25, 26], goldenrod ball-gall fly [27, 28]). Perhaps unsurprisingly, nearly all studies of HAD have emphasized insect responses to differences in plant traits between the alternative hosts, while downplaying variation in plant traits among individuals within each host. Such an interspecific perspective is obviously appropriate for studies using population-genetic tools to detect host-associated forms and reconstruct their history (e.g., [13, 29–31]), but it is also near universal in studies discussing ecological mechanisms by which host shifts and HAD proceed (e.g., [25, 26, 28, 32–38]). An alternative approach would explicitly recognize within-species variation in host-plant traits and consider possible roles for such variation in favouring or impeding host shifting and HAD. This approach has yet to be applied in earnest to any system, but intriguing hints at its usefulness appear in the literature for the goldenrod ball-gall fly, *Eurosta solidaginis*, and its races on the goldenrods *Solidago altissima* and *S. gigantea*. For example, *Eurosta* of the *S. altissima* race prefer the largest ramets of their host [39], and since *S. gigantea* plants tend to be shorter when *Eurosta* oviposits [40], this preference might discourage host-choice errors by *altissima* flies. In contrast, if *gigantea* flies similarly prefer taller ramets, they could be susceptible to host-choice errors (although *gigantea* flies’ preferences have not been assessed, and neither hypothesis raised here appears to have been tested). Work on the phenology of insect emergence and host-plant growth has similar implications. *Eurosta* adults emerge from *S. gigantea* earlier than from *S. altissima* [41], and this pattern is correlated with availability of rapidly growing ramets of each host to be attacked [40]. Thus, individual *S. altissima* ramets with earlier phenology, or *S. gigantea* ramets with later phenology, might be more likely to be attacked by the “wrong” host race. There is geographic variation in the abundance of such intermediate-phenology ramets, and How et al. [40] suggested that host shifts might be more easily initiated where host phenology overlaps more extensively.

Even for *Eurosta*, however, there are few plant traits for which insect responses have been studied on both alternative hosts, and so we know little about how insect responses to plant trait variation might relate to the ecology of host shifting and HAD. Furthermore, there is no system for which we can compare insect responses to plant trait variation for a set of insects attacking the same plants but differing in stage of host shifting and HAD. I outline here a conceptual model of host trait-space use during host shifting and HAD, along with a statistical approach for investigating trait-space use in phytophagous insects. I suggest hypotheses for temporal changes in host trait-space use over evolutionary time, from initial host-choice errors through to the independent evolution of a pair of well-isolated host-specialist sibling species. I call the overall model the “gape-and-pincher,” or “GAP,” model of trait-space use (the reason for this name will be apparent after the model is described). While I outline the model for plants and phytophagous insects, it will apply to many other systems with some straightforward vocabulary substitutions.

2. Conceptual “GAP” Model of Host Trait-Space Use

All plant species vary intraspecifically for numerous morphological, phenological, and chemical traits, with variation having genetic, epigenetic, and/or environmental causes (e.g., [42–45]). This variation defines a set of phenotypes that are available for attack by a phytophagous insect searching its environment for suitable hosts. This set of phenotypes can be depicted as a cloud of points in a multidimensional trait space, with each point representing an individual plant (or a ramet, for clonal plants; or even a module, when important variation occurs within individuals [46]). It is convenient to consider the two-dimensional case (Figure 1), which can represent either a system in which two plant traits show variation relevant to insect attack or a two-dimensional summary of a higher-dimensional trait space (using principal components to extract two dominant axes of trait variation). I use the term “available trait space” to describe this cloud of points, as combinations of plant traits falling inside it are available to attacking herbivores, while combinations outside are not available (i.e., they do not correspond to real plants which might be attacked). This trait space can be characterized by calculating its centroid (the point whose coordinate on each axis is the mean value of that coordinate for all individuals), its size (average distance from individual plants to the centroid), and its shape.

Consider first an insect interacting with a single host species. Some plant individuals will be attacked, but others will likely escape. The attacked individuals define the “attacked trait space” (filled circles in Figure 1), which must be a subset of the available trait space (and might be expected to be a smaller subset for more specialist herbivores [47, 48]). The relationship between available and attacked trait spaces will depend on active host-selection behavior by insects (insect preference), and also on whether insects can survive on an individual plant after initiating attack (insect performance). Both preference and performance will depend on plant traits—sometimes the same traits, but sometimes not. For simplicity, I use “herbivore attack” to denote the occurrence of feeding herbivores on plants, whether patterns in occurrence arise from preference or performance, and terms like “selective” attack should similarly be taken to include both preference and performance effects. The attacked trait space may represent a common preference by all herbivore individuals, or the sum of herbivore individuals’ distinct preferences in species with strong individual specialization [49, 50].
When herbivore attack is random with respect to plant traits (Figure 1(a)), the attacked trait space will resemble the available trait space in centroid location, shape, and (once corrected for the smaller number of attacked plants) size. For a selective herbivore (one that rejects some available plants), in contrast, the attacked and available trait spaces will differ. Many patterns are possible, but two are particularly likely. First, the herbivore might attack typical plants (those with trait values near the population means) and reject extreme ones, leading to an attacked trait space that is central with respect to the available trait space; the attacked space is smaller than the available space, but the two spaces have similar centroids (Figure 1(b)). Such a pattern might be favoured by selection because (for example) typical plants are most common, and insects preferring them pay lower search costs and experience greater resource availability. Alternatively, herbivore attack might be associated with extreme trait values (e.g., herbivores might perform best on the largest or least-defended individuals), leading to an attacked trait space that is marginal with respect to the available trait space: the attacked trait space is again restricted in size, but in this case the two spaces have different centroids (Figure 1(c)).

Now consider a pair of plant species available for attack (Extension to larger numbers of hosts is possibly but considerably complicating.) Given a common set of measured traits, we should see two clouds of points in trait space (Figure 2) defining a pair of available trait spaces. The distance between available trait spaces defined by different plant species might be large compared to the size of each available trait space [38], but, for closely related pairs of phenotypically variable plants, this need not be so (e.g., for *Solidago altissima* and *S. gigantea*, see Figure 3). Two available trait spaces could even be touching or interdigitated, especially for hybrid swarms [51]. One can again consider attacked trait spaces in comparison to available trait spaces on each host, but now there are many more possibilities, as each attacked trait space could be nonselective, central, or marginal (and if marginal, toward or away from the other host). Among possible patterns, I emphasize here a set of trait-space relationships predicted for an insect herbivore moving through a four-step evolutionary sequence: from original specialization on one of the two hosts, through a host shift, to early and late stages of HAD.

2.1. Stage 1: Single-Host Specialists and the Importance of Host-Choice Errors. An insect attacking a single host could show virtually any pattern in the relationship between attacked and available trait spaces, but some patterns are of special interest in the context of possible host-shifting to an evolutionarily novel host. (By a “host shift” I mean the addition of a novel plant to the herbivore species’ diet, which will normally occur without immediate abandonment of the old). Such host shifts are likely to begin when a few individuals attack the “wrong” (novel) host, making it possible for selection to favour the incorporation of the novel host into the insect’s host range. Importantly, the likelihood of host-choice errors is likely to depend on the insect’s use of plant trait space. In particular, imagine an insect showing a marginal attacked trait space on the ancestral host. That marginal attacked trait space could be adjacent to the available trait space defined by the novel host (Figure 2(a)), or could be distant from it. When it is adjacent, host-choice errors are more likely and insects making those errors are more likely to survive on the novel host [52]. In contrast, when the ancestrally attacked trait space is distant from the novel host, host-choice errors (and thus host shifting) should be less likely. I call this the “adjacent errors hypothesis.” The logic mirrors the widespread expectation that host-choice errors and host shifts are more likely between species that resemble each other morphologically, chemically, or phylogenetically [38] but stresses that the distance in trait space that needs to be crossed for a host-choice error depends not only on the distance between available trait spaces but also on how insect preference and performance define the attacked trait space.

2.2. Stage 2: Oligophagous Feeding Following Diet Expansion. Following a host shift that expands diet, our focal insect
species will be oligophagous, feeding on two hosts (ancestral plus novel) rather than one. Because this stage should follow from patterns in attack allowing host-choice errors (adjacent errors hypothesis), attacked trait space is likely to remain marginal on the ancestral host (Figure 2(a)). As host preference and performance evolve to include attack on the novel host, the attacked trait space on that host is likely to be marginal as well, but with the two attacked trait spaces adjacent (Figure 2(b)) because novel plants closer to the ancestrally attacked trait space are more easily colonized. I call this the “adjacent oligophagy hypothesis.”

Note that the adjacency pattern is equivalent to restricted but central use of an available trait space defined by the two hosts in combination (compare Figures 1(b) and 2(b)). If disruptive selection between alternative hosts does not act or is not powerful, oligophagy and the adjacency pattern could be evolutionarily persistent. Alternatively, this stage might be transient, persisting only until disruptive selection has
time to drive HAD of insect subpopulations exploiting the two hosts. The contrast between these possibilities highlights an important fork in the evolutionary road [53], in which disruptive selection favouring HAD is or is not sufficient to overcome gene flow working to homogenize the herbivore population and to maintain an oligophagous diet.

2.3. Stage 3: Nascent Host-Specialist Forms and the Selection-Gene Flow Tension. How might insect trait-space use favour or oppose the ability of disruptive selection to achieve HAD? Craig et al. [52] argued that persistent oligophagy is likely when the two available trait-spaces are very close, with HAD likely when they are more distant. However, their conceptual model assumes that attacked trait spaces on the two hosts remain indefinitely adjacent (their Figure 1). I suggest that there is another important possibility; a critical step in HAD may be the separation of the attacked trait spaces (Figure 2(c)) such that insect subpopulations on the two hosts (now appropriately thought of as nascent host forms) come to attack dissimilar individuals rather than similar ones. I call this the “trait distance-divergence hypothesis”. Separation of the two attacked trait spaces could arise in two different but complementary ways.

First, distance between attacked trait spaces could arise simply because disruptive selection for adaptation to the alternative hosts overpowers the homogenizing effect of gene flow between nascent host forms. Under this scenario, the attacked trait spaces could move to opposite ends of the available trait spaces, as in Figure 1(c), or merely further apart than adjacency, depending on the shape of the fitness landscape—that is, fitness optima on the alternative hosts might favour central or marginal trait spaces. Under this scenario, distance between attacked trait spaces is just a symptom by which the progress of HAD can be recognized.

Second, distance could be a product of selection to minimize host-choice errors by each nascent host form or hybridization between them [22]. Host-choice errors could be opposed by selection because they lead to preference-performance mismatches, or because they put larvae in competition with members of the other host form (encouraging divergence by character displacement). Alternatively, host-choice errors coupled with the tendency for phytophagous insects to mate on their host plants could lead to hybrid matings. Hybrid disadvantage is possible given tradeoffs in ability to exploit the alternative hosts, or if hybrids prefer or are best suited for trait-value combinations falling in the gap between the two available trait spaces (this gap is shown narrow in Figure 2 but will often be wider [38]). Selection to reduce hybridization by widening the distance between attacked trait spaces (Figure 1(c)) would be a form of reinforcement [54]. Under the reinforcement scenario, distance between attacked trait spaces is more than a symptom of HAD; once achieved, it serves to reduce gene flow between nascent host forms and permit HAD to progress.

In summary, the trait distance-divergence hypothesis holds that attainment of distance between attacked trait spaces (Figure 1(c)) can be both a symptom of HAD and also a factor permitting HAD. The larger the distance between attacked trait spaces, the more likely is the evolution of genetic differentiation between insects on the two hosts. Since genetic differentiation can ease the evolution of distance between attacked trait spaces, this stage of evolution can involve positive feedback [58–60]. In contrast, an insect for which attacked trait spaces remain adjacent (Figure 1(b)) is likely to remain an oligophagous insect with no host-associated structure to its gene pool.

2.4. Stage 4: Pair of Established Host Specialists. As HAD proceeds and gene flow between nascent host forms declines, we would expect the gradual accumulation of more, and more effective, reproductive isolating mechanisms [24, 61, 62]. This should continue until ecological speciation is complete, and the two host-specialist forms attain the status of full biological species. As reproductive isolation becomes enforced by multiple, redundant mechanisms, the importance of separation between attacked trait spaces should decline. Selection will then be free to mould trait-space use independently for each species, and if reinforcement earlier in HAD pushed the attacked trait spaces apart (trait distance-divergence hypothesis), this force can now relax. If selection favours use of central trait space on each host, for example (Figure 2(d); or if it favours nonselective use of trait space on each host), the distance between the attacked trait spaces should decrease. I call this the “distance relaxation hypothesis.”

Note that through the temporal sequence (Figures 2(a)–2(d)), the overall conceptual model suggests a pair of attacked trait spaces that begin close together, move apart, and then move back together like pincers. This movement underlies the terminology “gape-and-pincher model” of trait-space use.

2.5. What about Generalists? The foregoing considered insects that begin as monophagous on one of the two hosts and remain narrowly oligophagous or monophagous at all stages of HAD. However, many herbivores are broader generalists [63] for which we would not expect any of the trait-space patterns shown in Figure 2. In particular, it would be very surprising if a broad generalist showed nonrandom separation between attacked trait spaces (Figures 2(c) and 2(d)). Instead, attacked trait space might be nonselective on both hosts [47, 48], or restricted but marginal along a trait axis orthogonal to the difference between the two hosts (e.g., insects might prefer larger individuals of each host and also attack other, larger species). Such broad generalists are much less likely than host specialists to undergo HAD because (being already adapted to multiple hosts) they are less likely to experience strongly disruptive selection for performance on one host versus another [64].

2.6. Testing the Hypotheses. The four hypotheses that make up the GAP model are logically distinct; finding that one
Table 1: Relationships between patterns in trait-space use (Figures 2(a)–2(d)), the GAP model, and statistical tests implemented for analysis of attacked trait spaces.

<table>
<thead>
<tr>
<th>Pattern in trait-space use</th>
<th>Hypothesis</th>
<th>Attacked trait spaces</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>
| Monophagous, attacked trait space marginal and adjacent to alternative host (Figure 2(a)) | Adjacency favours host shifting (adjacent errors hypothesis) | Ancestral host: marginal | Novel host: marginal but rare | — | Attacked spaces close
| Oligophagous, attacked trait spaces marginal and adjacent (Figure 2(b)) | Adjacency persists after host shifting (adjacent oligophagy hypothesis) | Ancestral host: marginal | Novel host: marginal | — | Attacked spaces close
| Nascent host races, attacked trait spaces marginal and distant (Figure 2(c)) | Distance permits, and is also symptomatic of, genetic isolation (trait distance-divergence hypothesis) | Ancestral host: marginal | Novel host: marginal | — | Attacked spaces distant
| Pair of monophagous species, attacked trait spaces central on each host (Figure 2(d)) | Other isolating mechanisms reduce importance of trait-space distance (distance relaxation hypothesis) | Ancestral host: not marginal | Novel host: not marginal | Ancestral host: restricted (central) or nonselective | Attacked spaces neither close nor distant

1More strictly, only restricted or marginal trait-space use along the PC axis (or axes) defining the difference between available hosts is directly relevant to the GAP model.

2But statistical detection is difficult because attack on the novel host is rare.

hypothesis holds (or fails) implies nothing about the others. For example, for a given herbivore, the adjacent errors and adjacent oligophagy hypotheses could hold, but the distance-divergence and distance relaxation hypotheses fail, if HAD proceeds to ecological speciation without any movement of attacked trait spaces away from each other following the host shift.

Each of the four hypotheses can also be posed, and tested, at two levels. First, we can test each hypothesis for a single herbivore. For instance, are attacked trait spaces adjacent on Solidago altissima and S. gigantea for the narrowly oligophagous Epiblema scudderiana (adjacent oligophagy hypothesis)? Of course, such tests focus on patterns, and confirmation of a pattern need not constitute a strong test of underlying mechanism. Second, and more powerfully, we can test each hypothesis for herbivorous insects as a class. For instance, are attacked trait spaces (statistically) further apart for recently divergent and incompletely isolated pairs of host races than they are for more ancient specialist species pairs (distance relaxation hypothesis)? At this level, the hypotheses can hold strongly or weakly (or not at all); that is, the empirical relationship between trait-space distance and extent of reproductive isolation could be stronger or weaker (or non-significant).

Testing the adjacent errors, adjacent oligophagy, trait distance-divergence, and/or distance relaxation hypotheses for individual herbivores will require trait-space use data for large numbers of individuals on the alternative hosts. Some tests will be difficult at the individual-herbivore level (e.g., testing the distance relaxation hypothesis for an individual herbivore would require historical data on past trait-space use, which will only rarely be available). Ultimately, though, assessment of the GAP model depends more on comparative tests of the hypotheses for herbivores as a class, and this will require data on host trait-space use for multiple insect herbivores differing in host range. A particularly revealing approach will involve sets of herbivores that differ in the extent of their progression through HAD (e.g., [13]), because of the expectation that the trait-space relationships shown in Figures 2(a)–2(d) form a temporal evolutionary sequence. The cleanest comparative tests will involve herbivores sharing a common pair of alternative hosts, so that attacked trait spaces can be contrasted among herbivores (e.g., recent host forms and ancient specialist pairs, for the distance relaxation hypothesis) while seen against the simple backdrop of a common pair of available trait spaces.

Progress towards understanding the influence of trait-space use on the evolutionary trajectory of herbivore specialization, then, can be made by measuring for multiple insect herbivores the relationships between available and attacked trait spaces on the alternative hosts, for comparison with those suggested by the four hypotheses (summarized in Table 1). In particular, we will be interested in whether attacked trait space on each host is marginal (and in what direction), and whether the two attacked trait spaces are adjacent or distant.

Unfortunately, we do not yet have sufficient comparative data to test the GAP model. Members of my laboratory are beginning to gather such data for insect herbivores attacking the goldenrods Solidago altissima and S. gigantea. In following sections, I provide formal statistical tools for analysis of such data and apply them to an illustrative data set demonstrating a path towards the comparative hypothesis tests that are our ultimate goal.
3. Statistical Methods

I developed statistical methods to test for three patterns in host trait-space use by insect herbivores. These patterns are predicted, in different combinations, by the adjacent errors, adjacent oligophagy, distance-divergence, and distance relaxation hypotheses (Table 1) and thus provide windows on the overall GAP model. The tests share a common framework in that they are based on relationships between attacked and available trait spaces for host plants of two species (Figures 1 and 2). Two tests pertain to the pattern of attack on a single host, and the third to the pattern of attack on each host relative to the other.

First, I test for central versus marginal location of the attacked trait space on each host ("Marginal trait-space test"). I calculate the centroid of the available trait space (mean PC1 and PC2 scores for all available plants, attacked and unattacked) and that of the attacked trait space (mean PC scores for attacked plants only). I then calculate the distance between available and attacked centroids and compare this to a null distribution of 10,000 such distances calculated following random shuffling of attack status across all plant individuals. The fraction of randomization distances larger than the actual attacked-available distance is a \( P \) value, and when it is small we reject the null hypothesis that attacked and available plants have a common centroid (central or nonselective attack, Figures 1(a) and 1(b)) in favour of the alternative of marginal attack (Figure 1(c)).

When we are unable to detect marginal use of available trait space, we might seek to distinguish between nonselective (Figure 1(a)) and restricted but central (Figure 1(b)) alternatives. To do so, I use the "restricted trait-space test." I calculate the Euclidean distance from each attacked plant to the centroid of attacked trait space. The size of the attacked trait space is given by the sum of these distances. I then compare this trait-space size to a null distribution the sizes of 10,000 attacked trait spaces generated by randomly shuffling attack status across all plant individuals. Note that shuffling attack status maintains the number of attacked plants, which is critical when calculating the size of a trait space. The fraction of randomization attacked trait spaces smaller than the actual one is a \( P \) value, and when it is small we reject the null hypothesis that attack is nonselective. Since we are using the restricted trait-space test following a nonsignificant marginal trait-space test, the alternative is that herbivores exploit a restricted but central subset of available trait space.

Finally, I test whether the distance between attacked centroids on the two host plants is smaller or larger than expected at random ("Distant trait-spaces test," Figure 2(b) versus 2(c)). I first calculate the distance between attacked trait-space centroids on the two alternative hosts. This distance is compared, in a two-tailed test, to a null distribution of 10,000 such distances calculated following random shuffling of attack status across individuals of each plant species (separately). When the actual centroids are farther apart than the mean distance from randomizations, then twice the fraction of randomization distances that are larger than the actual distance is a \( P \) value, which when small supports rejection of the null hypothesis in favour of the alternative that the two attacked trait spaces are significantly distant. On the other hand, when the actual centroids are closer than the mean distance from randomizations, then twice the fraction of randomization distances that are smaller than the actual distance is a \( P \) value, which when small supports rejection of the null hypothesis in favour of the alternative that the two attacked trait spaces are significantly adjacent.

The marginal trait-space, restricted trait-space, and distant trait-space tests are implemented in TraitSpaces 1.20, a program written in Microsoft Visual Basic.NET for Windows. The software takes as input a datafile with a row for each individual host plant, and columns for host species identity, presence/absence of each herbivore, and first and second principal components calculated from the host trait matrix. (Principal components may be output from any standard statistical package.) Extension to trait spaces of higher dimensionality, if desired, is straightforward; one could even use an unreduced trait matrix at the cost of some complexity in displaying results. The analytical framework easily accommodates data for other host/attacker systems and could even be applied to cases where consumers use variable microhabitats or food resources. The current version of the TraitSpaces package is available from the author on request.

4. Field Methods

4.1. Study System. The goldenrods Solidago altissima L. and S. gigantea Ait. are clonal perennials codistributed over much of eastern and central North America. Intermixed stands of the two species are common in open habitats such as prairies, old fields, roadsides, and forest edges. Individual ramets grow in spring from underground rhizomes, flower in late summer and fall, and die back to ground level before winter. The two species differ most obviously in pubescence [65]: S. altissima stems are sparsely to densely short-hairy, especially basally, while S. gigantea stems are typically glabrous. Both species display extensive intraspecific variation (genetic and plastic) in most traits, including ramet size, pubescence, leaf shape, size, and toothiness, and chemical profiles ([27, 66, 67], S. B. Heard, unpubl. data).

S. altissima and S. gigantea are attacked by a diverse fauna of insect herbivores [68–70], which vary in diet specialization. Some are broad generalists that accept Solidago as part of a taxonomically diverse diet (e.g., the exotic spittlebug Philaenus spumarius [71]), and some are broadly oligophagous, feeding on Solidago among other members of the Asteraceae (e.g., the chrysomelid Exema canadensis [72]). Others are more narrowly oligophagous, attacking only Solidago spp. (e.g., the tortricid stem-galler Epiblema scudderiana [13, 73]). Finally, at least four herbivores have evolved monophagous host races or cryptic species on S. altissima and S. gigantea [13, 30, 74, 75], with divergence ranging from quite recent for the ball-gall fly Eurosta solidaginis (at most 200,000 years, but likely much less) to \( 2 \times 10^6 \) years old for the bunch-gall flies Rhopalomyia solidaginis/R. capitata.

Especially for the better-studied S. altissima, attack by various herbivores is known to vary among clones [68, 76, 77], and with plant traits including ramet size [39, 78],
growth rate [79], nutritional status [80, 81] and ploidy where this varies locally [82]. These trait-attack relationships involve both plant resistance and insect preference [27] and may be concordant or discordant among different herbivore species [68, 82].

4.2. Field Data. I and my field team gathered data on plant traits and herbivore attack in old-field and trailside Solidago populations in Fredericton, NB, Canada (45° 57′ 30″ N, 66° 37′ 1–20″′ W). Here both Solidago altissima and S. gigantea are abundant along with S. rugosa, S. junccea, S. canadensis, Euthamia graminifolia, Symphyotrichum spp., and other Asteraceae. S. altissima is exclusively hexaploid in the east, and S. gigantea exclusively diploid, so effects of ploidy on herbivore attack [83] need not be considered here.

It is important to assess the available and attacked trait spaces using traits measured before herbivore attack; otherwise, herbivore responses to plant traits could be confounded with herbivore-driven changes in the same traits. In early June 2004, we marked 104 S. altissima ramets and 186 S. gigantea ramets by setting line transects through well-mixed patches of the two species and marking each ramet touched by the line. At the time of marking, a few ramets had already been attacked by the stem-galler Gnorimoschema gallaesolidaginis (Lepidoptera: Gelechiidae; galls on 4 S. altissima and 3 S. gigantea ramets), but other herbivores

![Figure 3: Attacked trait spaces for four goldenrod herbivores on S. altissima (triangles) and S. gigantea (circles). Filled symbols denote attacked plants, and open symbols unattacked ones. Axis labels are shorthand for the first two principal components from a 7-variable morphological dataset; full factor loadings are provided in Table 2.](image-url)
had yet to attack. We measured 7 morphological traits of our marked ramets, focusing on easily measured traits that were likely to influence herbivore attack, that help distinguish the two study species, or both. We measured stem trichome density by counting, in the field with a hand lens, all trichomes in silhouette along a 10 cm length of stem just below the terminal bud. We measured stem width 5–10 cm above ground using a caliper, and stem height (from ground to the base of the terminal bud) using a measuring tape. For the largest leaf from each ramet, we measured leaf length, leaf width at the widest point, and the number of teeth along one leaf edge. Finally, we weighed each largest leaf before and after drying to constant mass at 45-55°C and calculated percent water content.

We surveyed marked ramets twice weekly until the end of August, identifying herbivores present as specifically as possible without disturbing them on the plant (for some groups, like larval Trirhabda beetles, species-level identifications require the removal of the insects to the laboratory, and we wanted to leave plants to experience natural levels of herbivory). When herbivores of the same species were present on consecutive surveys, we were usually unable to determine whether they were the same individuals, so rather than count individuals we classified each ramet as attacked or unattacked by each herbivore over the course of the entire season.

Some marked ramets were lost or damaged during the season, leaving 92 S. altissima ramets and 175 S. gigantea ramets with comprehensive herbivory and plant-trait data. Four herbivores were identifiable to species and abundant enough to give our analyses reasonable power: the xylem-sucking spittlebug Philaenus spumarius, which is broadly polyphagous [71]; the folivorous chrysomelid beetle Exema canadensis, which is oligophagous with many hosts in the tribe Astereae [72]; the phloem-sucking aphid Uroleucon nigrotuberculatum, which is narrowly oligophagous on Solidago spp. [84]; and the gall-making cecidomyiid fly Rhopalomyia solidaginis/R. capitata, which is a pair of monophagous specialists (R. solidaginis on S. altissima and R. capitata on S. gigantea [13]). All further analyses use this reduced set of 267 ramets and 4 herbivores.

### Table 2: Correlations among morphological variables measured for S. altissima and S. gigantea.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Loadings on PC1</th>
<th>Loadings on PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem width</td>
<td>0.88</td>
<td>0.72</td>
</tr>
<tr>
<td>Ramet height</td>
<td>0.75</td>
<td>0.47</td>
</tr>
<tr>
<td>Leaf length</td>
<td>0.18</td>
<td>0.40</td>
</tr>
<tr>
<td>Leaf width</td>
<td>0.47</td>
<td>0.56</td>
</tr>
<tr>
<td>Teeth</td>
<td>0.32</td>
<td>0.24</td>
</tr>
<tr>
<td>Water content</td>
<td>-0.18</td>
<td>-0.01</td>
</tr>
<tr>
<td>Trichomes</td>
<td>-0.48</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Factor loadings for the first two principal components from the morphological data matrix for S. altissima and S. gigantea.

5. **Field Results and Discussion**

#### 5.1. Plant Traits.

Among the 7 measured traits stem width, ramet height, and leaf length were strongly intercorrelated ($0.72 < r < 0.88$), suggesting that all three reflect overall ramet size. The other 18 correlations were weak to moderate (Table 2). The first two principal component axes explained 47% and 28% of the morphological variance (75% total), while no other axis explained more than 9.3%. PC1 largely reflects ramet size (heavy loadings for stem width, ramet height, and leaf length; Table 3), but also leaf toothiness (positively) and water content (negatively). PC2 contrasts trichome counts (strong positive loading; Table 3) with leaf width (strong negative loading) but also includes leaf water content, leaf length, and toothiness (all negative). These two principal components do a good job of capturing both intraspecific and interspecific variation (Figure 3), with S. altissima and S. gigantea separated primarily along PC2 (the pubescent S. altissima with high scores, and the glabrous S. gigantea with low scores).

#### 5.2. Herbivore Use of Phenotype Space.

Attack rates by Solidago herbivores are generally low (often 1–10% or even less), with the exception of some diet generalists and outbreaking species in high-density years (S. Heard, unpubl. data). In our dataset, even though we worked with some of the most common herbivores, only one herbivore on one host had an incidence above 30% (Exema canadensis on S. altissima, 64% of ramets attacked). Other herbivore/host combinations had lower incidences, with several less than 10% (Uroleucon nigrotuberculatum on both hosts and Rhopalomyia capitata on S. gigantea; Table 4).

The patterns I document in trait-space use could have arisen via herbivore preference, or via performance if poor herbivore growth leads to death or departure of herbivores before surveys can detect them. For most herbivores, repeated surveys allow herbivore detection shortly after attack begins, and so preference is the most likely driver of patterns in attack. However, for gallmakers like Rhopalomyia performance at the stage of gall induction could be important.
Plant genotype effects on gall induction, mismatched with herbivore preference, are known (for example) for *Eurosta solidaginis* on *Solidago altissima* [85].

The two most generalist herbivores (*Philaenus* and *Exema*) showed similar patterns in trait-space use (Figures 3(a) and 3(b); Table 4). Both showed significant evidence for nonrandom use of available hosts (marginal trait-space test). For *Philaenus* on both hosts, attack was concentrated on larger but less pubescent ramets (higher PC1 and lower PC2), while for *Exema* on both hosts attack was concentrated on larger ramets but did not depend on pubescence (higher PC1). For both species, the distance between attacked trait-spaces on *S. altissima* and *S. gigantea* was slightly but not significantly smaller than expected under the null (distant trait-spaces test).

For the oligophagous *Uroleucon* (Figure 3(c)), attack on both hosts was significantly marginal, being concentrated on larger and less pubescent ramets. The distance between the two attacked trait-spaces was slightly, but not significantly, larger than expected under the null. Because this herbivore had the smallest sample size (just 17 attacked ramets total), these tests have much less power than for the more common generalists.

For the monophagous *Rhopalomyia* species pair (Figure 3(d)), there was no evidence on either host for marginal use of trait space, and the restricted trait space test suggests nonselcetive rather than central use of available trait space (Table 4). The distance between the two attacked trait-spaces was slightly, but not significantly, larger than expected under the null. Sample size, however, was very small for *R. capitata* on *S. gigantea* (8 attacked ramets), so the tests for that species and for the distance between attacked centroids are likely not very powerful.

### 5.3. Interpretation and Prospects.

The clearest pattern in the illustrative dataset is that for three of four herbivores, attack is significantly concentrated on larger ramets (large PC1). The stem gallers *Eurosta* [39] and *Gnorimoschema* [78] also have well-documented associations with larger ramets, something that is common but not universal among phytophagous insects [86, 87]. Such concordance across herbivore species in the use of trait space increases the likelihood of multiple herbivores cooccurring on a single ramet—something very unlikely under the null hypothesis of independent occurrence, since most attack rates are low. Herbivores cooccurring on a plant may compete directly (via resource consumption) or indirectly (via induced resistance) or may even show facilitation [88] although we know little about potential interactions among goldenrod herbivores [89–91]. However, concordance among goldenrod herbivores in use of plant trait space is far from universal [68, 82].

How do the illustrative data fit with the GAP model of trait space use during host shifting and HAD laid out above? The tendency for most herbivores to attack larger ramets (larger PC1) generates pattern in trait-space use. However, this shared tendency means that both attacked trait spaces are offset from the available spaces, in parallel and orthogonally to the contrast between alternative host plants (PC2). The distance between attacked trait spaces is unaffected, and so this ramet-size pattern is not directly relevant to the GAP model. Three of the four herbivores analyzed (*Philaenus*, *Exema*, and *Uroleucon*) have host ranges broader than just the *S. altissima*-S. gigantea pair and might therefore be expected to be rather unselective about traits distinguishing the two hosts. Indeed, *Exema* showed no offsets between attacked and available trait spaces along the principal components axis contrasting *S. altissima* and *S. gigantea* (PC2; Table 4). *Philaenus* and *Uroleucon* did show offsets along this axis, but because they were in the same direction and roughly equal on the two hosts, separation of attacked trait-spaces was not significantly large for either herbivore (Table 4, distant trait spaces test). The fourth herbivore, *Rhopalomyia*, is a pair of relatively old monophagous species [13]. For such a species pair, the distance relaxation hypothesis suggests that the use of distant trait spaces may no longer be an important barrier to gene flow (Figure 2(d)). *Rhopalomyia*’s use of trait space

### Table 4: Tests of trait-space use on *Solidago altissima* and *S. gigantea* for four *Solidago* herbivores. *P*-values in bold are significant at \( \alpha = 0.05 \).

<table>
<thead>
<tr>
<th>Herbivore</th>
<th>Host</th>
<th># Attacked plants</th>
<th>Distance from available centroid</th>
<th>Marginal trait-space test ( P )</th>
<th>Restricted trait-space test ( P )</th>
<th>Distance between attacked trait-spaces ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PC1</td>
<td>PC2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Philaenus</em></td>
<td><em>S. altissima</em></td>
<td>25</td>
<td>1.00</td>
<td>-0.50</td>
<td>&lt;0.001</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>S. gigantea</em></td>
<td>22</td>
<td>1.11</td>
<td>-0.52</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td><em>Exema</em></td>
<td><em>S. altissima</em></td>
<td>59</td>
<td>0.30</td>
<td>0.05</td>
<td>0.023</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>S. gigantea</em></td>
<td>33</td>
<td>0.65</td>
<td>0.04</td>
<td>0.023</td>
<td>—</td>
</tr>
<tr>
<td><em>Uroleucon</em></td>
<td><em>S. altissima</em></td>
<td>9</td>
<td>1.39</td>
<td>-0.10</td>
<td>0.007</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>S. gigantea</em></td>
<td>8</td>
<td>2.32</td>
<td>-0.62</td>
<td>&lt;0.001</td>
<td>—</td>
</tr>
<tr>
<td><em>Rhopalomyia</em></td>
<td><em>S. altissima</em></td>
<td>27</td>
<td>0.09</td>
<td>0.35</td>
<td>0.21</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td><em>S. gigantea</em></td>
<td>8</td>
<td>0.66</td>
<td>-0.05</td>
<td>0.31</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1. Of 92 available *S. altissima* and 175 available *S. gigantea* ramets.
2. Attacked centroid minus available centroid (PC1 and PC2 components). A positive entry means that ramets with a large PC score are more likely to be attacked.
3. “Small” if the two attacked trait spaces are adjacent (Figure 2(b)), and “large” if the two attacked trait spaces are distant (Figure 2(c)).
4. This test is informative only when the marginal trait-space test is not significant.

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[85] A. J. Wolfe. *Gall wasps*—*S. altissima* and *S. gigantea*.- *S. altissima* and *S. gigantea*...
(Table 4: no evidence for marginal attack, and separation between the two attacked trait spaces no larger than expected at random) is consistent with this (Table 1). Overall, none of the illustrative data are inconsistent with the GAP model, but none of the four herbivores analyzed provides a strong test of its hypotheses. I did not find any examples of the patterns hypothesized for a single-host specialist making host-choice errors, for a narrowly oligophagous species immediately following a host shift, or for a pair of nascent forms early in HAD (Table 1, Figures 2(a)–2(c)). This is not surprising, though, because species known to be in early stages of HAD on S. altissima/S. gigantea, such as the ball-gall fly Eurosta solidaginis and the spindle-gall moth Gnorimoschema gallsolidaginis, were insufficiently abundant for analysis. At the broader level of hypothesis testing, four herbivores constitute just a small step towards assessing general patterns in trait-space use through HAD and ecological speciation. It will take many studies like mine, with herbivores on Solidago and other plants, before we can assess the generality of patterns in trait-space use. Because attack rates for most Solidago herbivores are low, achieving powerful hypothesis tests for any herbivore will entail marking very large numbers of ramets—especially since ramet selection must be done before attack begins to avoid distortions of attack-space measurements if trait values change under herbivore attack. In Solidago, for instance, ramet biomass and height are often reduced by herbivory [91–93]. I am currently expanding on the illustrative study with the goal of securing larger sample sizes for the herbivores studied here and acceptable sample sizes for many more herbivores.

Another obvious limitation of the illustrative dataset is that it includes measurements of only seven plant traits, and conspicuously omits leaf-chemistry traits (and ploidy [83], which varies elsewhere but not in New Brunswick). S. altissima and S. gigantea have complex secondary chemistry, and variation in leaf chemistry is known to influence herbivore attack [66, 67]. Unmeasured morphological traits may also be relevant to insect attack; for instance, Philaenus prefers plant species and individuals with wider leaf axils [94], and this trait varies among S. altissima genotypes (Maddox unpubl. in [77]). Expanding the list of measured traits, and especially incorporating leaf chemistry, is a high priority for future work.

Despite the small numbers of attacked ramets and measured traits that earn the “illustrative” dataset its descriptor, my analysis of trait-space use for four herbivores establishes that the field and analytical approach outlined here is feasible and can detect nonrandom trait-space use. Because the gold-enrod system includes such a diverse herbivore community attacking syntopic ramets of the alternative hosts, it offers the potential for great progress in testing hypotheses about host trait-space use during hostshifts and HAD.

6. General Discussion
The literature on how insect preference and performance vary with intraspecific variation in host-plant genotype, morphology, chemistry, and phenology is immense [95, 96]. Similarly, interspecific variation in the same kinds of traits has been widely held up as the key to the macroevolutionary fate of herbivore lineages (host shifting, diversification, specialization, and so forth [38, 63, 64, 97]). What is surprising is that the intersection of these perspectives is so little developed: we know almost nothing about trait-space use in systems where host shifting and HAD are suspected. This gap is clearly illustrated by the two best-studied cases of HAD in phytophagous insects: Eurosta solidaginis on Solidago altissima and S. gigantea and Rhagoletis pomonella on apple and hawthorn. For Eurosta, despite a wealth of information about how preference and performance relate to genetic and trait variation within S. altissima [27], few comparable data are available for flies attacking S. gigantea (except see [40]). For Rhagoletis, much has been written about the importance for HAD of apple-hawthorn differences in ripening phenology [26, 98, 99] and fruit size [100]. However, data on local intraspecific variation in phenology appear to be unavailable (although latitudinal clines have been documented [26]), and data on intraspecific fruit size variation appear limited to confirming significance of interspecific differences in average fruit size [100]. This is not to criticize work on these two model systems, which has pioneered the study of HAD, but rather to draw attention to a significant opportunity for progress.

Of course, the GAP model likely falls short of recognizing the full complexity of trait-space use in nature. While I have focused on snapshots of trait variation and insect use of trait space at a single site and in a single year, both available trait space and its use are likely to vary in space and time. This variation could have interesting and important consequences for HAD. For example, intraspecific variation in Solidago phenology and the difference in average phenology between S. altissima and S. gigantea change in space and time, and phenological differences are involved in host choice for at least two Solidago herbivores undergoing HAD (Eurosta [40]; Gnorimoschema gallsolidaginis, S. B. Heard, unpubl. data). Hawthorn phenologies show latitudinal gradients across space favouring local adaptation rather like that required during Rhagoletis’ host shift to apple [26]. There are thus likely to be places or times that are more conducive to host shifts and HAD than others [40, 52, 53]. Superimposed over this variation in available trait space can be strong geographic variation in insect preference (e.g., [82, 101]) and thus trait-space use. As a consequence, the places or times conducive to host shifting for one insect herbivore might not be so conducive for shifts by another. This is consistent with the evolutionary pattern seen in the Solidago system, in which three gallmakers have made host shifts from S. altissima to S. gigantea but have done so at different times [13].

Thinking about intraspecific variation in plant traits, and patterns of insect attack with respect to that variation, can expand and enhance our view of ecological speciation by phytophagous insects. Testing the hypotheses I frame about trait-space use for herbivores differing in diet breadth and in progress along the evolutionary sequence of HAD (Table 1) could take us a long way towards a predictive understanding of diet evolution and specialization in phytophagous insects. Ultimately, we would like to know for which taxa...
host-shifting and HAD are likely, and for which taxa they are not—and why [13, 38, 64, 102]. While much data collection and analysis lies ahead, the trait-space perspective promises a new and powerful window on the fascinating complexity of insect-plant interactions and herbivore diversification.

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References


Review Article

Habitat Choice and Speciation

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The role of habitat choice in reproductive isolation and ecological speciation has often been overlooked, despite acknowledgement of its ability to facilitate local adaptation. It can form part of the speciation process through various evolutionary mechanisms, yet where habitat choice has been included in models of ecological speciation little thought has been given to these underlying mechanisms. Here, we propose and describe three independent criteria underlying ten different evolutionary scenarios in which habitat choice may promote or maintain local adaptation. The scenarios are the result of all possible combinations of the independent criteria, providing a conceptual framework in which to discuss examples which illustrate each scenario. These examples show that the different roles of habitat choice in ecological speciation have rarely been effectively distinguished. Making such distinctions is an important challenge for the future, allowing better experimental design, stronger inferences and more meaningful comparisons among systems. We show some of the practical difficulties involved by reviewing the current evidence for the role of habitat choice in local adaptation and reproductive isolation in the intertidal gastropod Littorina saxatilis, a model system for the study of ecological speciation, assessing whether any of the proposed scenarios can be reliably distinguished, given current research.

1. Introduction

The role of divergent natural selection in speciation has been widely studied in recent years [1]. There is now broad acceptance that selection of this type can lead to the evolution of reproductive isolation, even in the face of gene flow [2]. Nevertheless, significant controversy remains. Is “ecological speciation” really distinct from other modes of speciation [3]? Why does reproductive isolation remain incomplete in some cases but not in others [4]? Do chromosomal rearrangements [5] or divergence hitchhiking [6] help to overcome the antagonism between selection and recombination? What is the role of the so-called “magic traits” [7]?

“Habitat isolation” is one part of the ecological barrier to gene exchange between species that includes effects due to local adaptation, competition, and choice [8]. In this paper, we will focus our attention on habitat choice, discussing the nature of its role in ecological speciation and the potential contribution towards reproductive isolation of various forms of habitat choice. We define habitat choice as any behaviour that causes an individual to spend more time in one habitat type than another compared with the expectation based on random dispersal (see “habitat selection”, p. 184, [9]). On the basis of this definition, a simple reduction in dispersal distance would not constitute habitat choice. Examples of mechanisms that might underlie choice include active movement into a preferred habitat; reduced dispersal in the preferred habitat relative to a nonpreferred habitat; preferential settling of propagules after a dispersal phase or a change in the timing of dispersal that influences the probability of arriving in a different habitat. Habitats may be spatially separated on various scales, from abutting distributions to a fine-scale mosaic, even different parts of the same host plant [10]. They also need not be separated in space at all: temporal or seasonal separation is possible. In the case of seasonal separation, allochronic emergence or reproduction [11] effectively constitutes forms of habitat choice but will not be considered here.
Habitat heterogeneity can lead to ecological speciation in the presence of gene flow. It requires divergent selection, which results in the establishment of a multiple-niche polymorphism [1] (Figure 1). This might arise *in situ* or on secondary contact between previously allopatric populations. A mechanism for nonrandom mating must then become associated with this polymorphism [12]. As a result of this two-step process, habitat choice can influence the probability of speciation or the degree of reproductive isolation achieved in one of two ways; firstly, habitat choice may increase the range of parameters under which a stable polymorphism can be maintained by selection in a heterogeneous environment [13]. This effect can be independent of any effect on mating pattern if, for example, individuals feed in two contrasting environments but mate away from the food resources. Secondly, habitat choice may cause assortative mating if it results in partially independent mating pools. Habitat choice of this type may be favoured by selection against hybrids and so constitutes a form of reinforcement [14]. Choice of mating habitat may cause assortment without influencing local adaptation but, clearly, habitat choice may have both effects (i.e., on polymorphism and assortative mating) simultaneously in some systems, strongly influencing the likelihood and speed of speciation [15].

Additionally, the trait responsible for habitat choice and the locally adapted trait—responsible for adaptations to local conditions—must generally become associated, typically requiring the build-up of linkage disequilibrium but potentially facilitated by pleiotropy (see preference-performance correlations in [16]), as is the case for other components of reproductive isolation [2]. However, it is possible for habitat choice to be under direct selection and contribute to nonrandom mating, thus constituting a “magic trait” (*sensu* Gavrilets [17]). *“Magic traits”* are usually discussed in the context of locally adaptive traits that also contribute to assortative mating, such as signals and preferences, though the concept can clearly be applied to any trait that promotes reproductive isolation. A recent attempt at clarification of the “magic trait” definition suggested “a trait subject to divergent selection and a trait contributing to nonrandom mating that are pleiotropic expressions of the same gene(s)” [7] but this view unfortunately confounds two distinct ideas: the impact of a single trait on more than one component of reproductive isolation and the effect of a gene on more than one trait. Here, we follow the proposal by Smadja and Butlin [2] to distinguish “single-effect” and “multiple-effect” traits and avoid the use of the, now confusing, term “magic.”

Multiple-effect traits facilitate the evolution of reproductive isolation by reducing or removing the need for linkage disequilibrium and so avoiding the negative effects of recombination [2, 18]. Their contribution to speciation still depends on the magnitude of their effects [19]. A trait that contributes to reproductive isolation through habitat choice may also contribute to reproductive isolation in other ways, including but not exclusively through effects that lead to direct selection, and so may be a multiple-effect trait. Other things being equal, we expect such traits to increase the probability or speed of speciation.

Habitat choice may also evolve by either a “one-allele” or a “two-allele” mechanism [18], as in the “single-variation” and “double-variation” models of de Meester et al. [20]. A “one-allele” scenario might involve “habitat matching” (e.g., [21]), for example, causing an animal to move until it is cryptic against its background and then to remain stationary. Indirect selection would favour the spread of such an allele in an environment with two backgrounds where predation maintained a polymorphism for alternate cryptic colours, without the need for linkage disequilibrium because the matching allele is favoured in association with both colour morphs. Alternatively, a “two-allele” mechanism might involve one allele favouring upward movement and another favouring downward movement. Here, the evolution of habitat choice, and so reproductive isolation, relies on linkage disequilibrium between the upward movement allele and alleles conferring increased fitness in the high habitat and between the downward allele and the alternate alleles at the fitness loci. Note that the “one-allele” versus “two-allele” distinction still holds for polygenic traits and can be made without knowing the genetic basis of the trait—the primary issue is whether the trait has to change in the same direction (one-allele) or in different directions (two-allele) in the diverging populations. “One-allele” mechanisms increase the probability of speciation and can potentially be more effective in limiting gene flow between subpopulations than two-allele traits. As with multiple-effect traits, this is because they remove the need for linkage disequilibrium.

Habitat choice may be plastic, including the possibility of learning, and plasticity can be an important factor in speciation [22]. The extent of plastic response may vary, up to the point where no genetic difference needs to exist between individuals showing behavioural preferences for contrasting environments. However, plasticity itself has a genetic basis; evolution can act upon the degree of plasticity, both the ability to learn and biases in the way individuals learn can evolve. Evolution of plasticity or learning may be considered examples of a “one-allele” mechanism, where

![Figure 1: Habitat choice and the probability of speciation. Darker colours represent increased probability of speciation. See main text for further explanation.](image-url)
the ability to modify the learning phenotype in a way that results in advantageous habitat choice is genetically determined and the same alleles, for effective learning, are beneficial everywhere.

These three different criteria for classifying traits responsible for habitat choice (whether habitat choice influences the maintenance of polymorphism or assortative mating; whether habitat choice traits are multiple or single effect; whether they follow a “one-allele” or “two-allele” inheritance mechanism) are largely independent. An exception arises because habitat choice may influence both the maintenance of polymorphism and assortative mating, and this is an example of a multiple-effect trait. If all possible combinations between the three criteria are considered, they may therefore lead, at least theoretically, to ten different habitat choice scenarios (Figure 1). Here we will discuss the implications of each one of these criteria for habitat choice and speciation, including examples of empirical studies and/or models. We will also review and discuss the current evidence for the role of habitat choice in local adaptation, including its possible effect on ecological speciation in an intertidal gastropod, the rough periwinkle *Littorina saxatilis*. We use this example to illustrate some of the practical difficulties involved in distinguishing among habitat choice scenarios empirically. This system also emphasizes the generality of the distinctions by comparison with the more widely studied insect models in which host plants form the contrasting habitats.

2. Habitat Choice Scenarios

Figure 1 provides a conceptual classification of habitat choice scenarios, illustrating the likelihood of progression towards speciation under each scenario (a scenario consisting of a combination of criteria). It suggests that, at one extreme, habitat choice may contribute to the maintenance of polymorphism without directly contributing towards reproductive isolation (as indicated by the lightest area) whereas, at the other extreme, habitat choice evolution may be a primary driver of speciation (as indicated by the darkest, central areas). For simplicity we have erected a categorical system (our “criteria”). However, due to the dynamic nature of the evolutionary process, one or more habitat choice scenarios from our framework may contribute to speciation at different stages in the process. For example, stabilisation of multiple-niche polymorphism may often be an early stage of speciation whereas the evolution of divergent preferences for mating habitat could be a form of reinforcement at a late stage of the process. Below, we will discuss the relevance of each criterion of this classification with reference to illustrative examples. However, it will quickly become clear that there is often insufficient empirical information to be certain how individual case studies fit into our classification, partly because the role of habitat choice in speciation has not been addressed in a strong conceptual framework. Additionally, we were unable to identify good empirical examples that satisfactorily demonstrate some of our proposed scenarios—this highlights both the limited range of the studies that have been undertaken in this area and the difficulty in identifying the mechanisms involved. We illustrate some of these difficulties in a final section dealing with one particular example, the periwinkle *Littorina saxatilis*.

2.1. Polymorphism or Nonrandom Mating. The first of our categories deals with the role of polymorphism and nonrandom mating in habitat choice scenarios: the establishment of polymorphism generated by divergent selection is a necessary step in ecological speciation.

Ecological speciation in the presence of gene flow requires divergent selection, which results in the establishment of polymorphism [1]. The maintenance of genetic variation within populations in heterogeneous environments has been widely discussed in the past, from theoretical models to experimental studies (reviewed in [23]). One of the first models, by Levene [24], showed that the maintenance of polymorphism in an environment with two habitats was possible. In this model there is random migration of individuals into the habitats, selection favours one of the genotypes in each habitat, each habitat contributes a constant number of individuals to the next generation, and there is random mating. The range of the parameters necessary to maintain polymorphism is rather restricted; nevertheless, this model was the basis for Maynard Smith’s [25] classic analysis of sympatric speciation. It is clear that under this model habitat choice is favoured by indirect selection (local adaptation to one of the habitats will secondarily promote preference for that habitat) and that habitat choice considerably expands the range of parameters under which the polymorphism is stable. Many subsequent models have shown that frequency- or density-dependent selection makes polymorphism more likely (e.g., [26, 27]) and so extends the range of situations in which an initial polymorphism will create conditions for the evolution of habitat choice, or where preexisting habitat choice will favour the establishment of multiple-niche polymorphism, reinforcing local adaptation (e.g., [20, 28]). The interplay between habitat choice and local adaptation has recently been reviewed by Ravigne et al. [13, 29]. From the point of view of speciation, the maintenance of local adaptation creates opportunities for the further evolution of reproductive isolation [25]. Therefore, if habitat choice helps to maintain polymorphism and if polymorphism reinforces local adaptation, it also increases the likelihood of speciation. This is true even if habitat choice has no influence on mating pattern. However, if habitat choice also generates assortative mating, its contribution to speciation will be greater (Figure 1). Habitat choice of this type is favoured by indirect selection when a polymorphism is established and this constitutes a form of reinforcement [14]. Finally, it is possible that habitat choice may apply only to the mating habitat and so influence mating patterns without being directly connected to a source of divergent selection. To understand the role of habitat choice in speciation, it is important to distinguish these categories and to determine the stage in the speciation process at which choice evolves.

Natural cases where both polymorphism and habitat choice are present in the population (but the choice does not influence mating pattern) are uncommon in the literature.
From the point of view of speciation, these examples should represent initial stages of speciation or divergence, but the outcome of this process is highly dependent on the reigning conditions (e.g., habitat distribution, habitat size, and selection coefficients) and there may actually be no progression towards greater isolation [4]. The most clear-cut examples are likely to come from species where mating occurs in a different habitat from the majority of the lifecycle, such as aquatic insects with a brief aerial mating phase. One interesting possible case concerns the aphid genus Cryptomyzus in which sibling species, which still occasionally hybridise in the field, utilise either dead nettle (Lamium gale-obololon) or hemp-nettle (Galeopsis tetrahit) as summer host. On these hosts they reproduce asexually, but their sexual generations occur on redcurrant (Ribes rubrum) regardless of the summer host [30]. Here, there is assortative mating but it is not due to the strong preference for distinct summer hosts. Rather, it seems to be mediated by differences in the diurnal cycle of pheromone release by females. This situation, where multiple races or species share the same primary host while utilising distinct summer hosts, seems to be common in aphids. The scenario where habitat choice can maintain a polymorphism but have no influence on nonrandom mating appears to be rare but may simply be under-represented in the literature. It is not likely to be favourable for progression to complete reproductive isolation.

The opposite situation, where habitat choice influences mating alone, is also not widely documented but is also most likely where mating occurs in a distinct habitat from the majority of the life cycle. A possible example may occur in the mosquito Anopheles gambiae whose larvae develop in small, often temporary water bodies but whose adults mate in aerial swarms. The M and S molecular forms of A. gambiae cooccur in many parts of Africa and show strong, but incomplete reproductive isolation. A major contributor to pre-zygotic isolation appears to be the choice of distinct habitats in which to form mating swarms [31]. This scenario is also unfavourable for speciation because there is no close connection between assortative mating and a source of divergent selection.

In the majority of cases habitat choice is likely to influence both maintenance of polymorphism and assortative mating, as mating usually takes place in the same habitat as the life-cycle phase in which selection occurs. A trait responsible for habitat choice of this type may be considered a multiple-effect trait because it has effects on reproductive isolation both through assortative mating and through enhancing local adaptation. This combination may generate a higher probability of progressing to complete reproductive isolation than cases where habitat choice influences only one component of isolation. The situation is typical of many phytophagous insects, which remain on their host to mate, and has been very widely studied in this context [32], including important early models (e.g., [28]) and classic research on the apple maggot fly, Rhagoletis pomonella. Sympatric host races of the apple maggot fly in North America have evolved in the last 150 years, with a host shift occurring from hawthorn to apple around 1860 [33]. Life cycles in these two host races are very similar; mating occurs on the plant and females oviposit in ripening fruit. Larvae complete their development in the ripe fallen fruits, pupate in the soil, and undergo a facultative diapause until spring. Adults congregate again on the host plant for mating. Host races are differentially adapted, primarily through their diapause characteristics which match the timing of the lifecycle to differences in phenology of their hosts [34, 35]. There are also host-associated differences in larval survival [36].

Host fidelity in Rhagoletis is partly a result of limited dispersal but there is clear evidence for active habitat choice involving fruit size and colour and, especially, volatile chemical signatures [37, 38]. Because there are clear fitness differences and mating occurs on the host fruit, host choice clearly contributes to both stabilisation of the coexistence of the races and to assortative mating between them.

The historical role of host choice is less easily determined. Feder [39] suggested that individuals with a genetic preference for apples, the derived host, may have gained an immediate selective advantage, perhaps involving the use of an empty niche or escape from parasitism (“apple race flies have less parasitoids than hawthorn race flies because parasitoids use plant cues when searching for their hosts,” [40]). In this case, habitat choice may have evolved first and facilitated subsequent host adaptation, rather than evolving in response to the fitness costs of oviposition on the wrong host. Habitat choice would then be a multiple-effect trait since it would be under direct selection, as well as contributing to assortative mating (see below). A further complication to this hypothesis is the association of some host-specific traits with chromosomal inversions. These inversions appear to predate the introduction of apples to North America and may have evolved during a period of allopatry [41]. Their presence in the population may also have facilitated the host shift, interacting with changes in preference.

2.2. Habitat Choice: “Multiple-Effect Trait” versus “Single-Effect Trait”. The Rhagoletis example nicely illustrates the distinction between multiple-effect and single-effect traits, as defined in Section 1. We can envisage two possible historical sequences. In the first, multiple-effect, scenario, an allele arose in the ancestral hawthorn population which increased the likelihood of females ovipositing on apple. This habitat choice allele was favoured by direct selection because larvae developing on apple had higher survival but at the same time contributed to isolation through its impact on assortative mating. This led to the establishment of a population on apple trees, which further adapted to the new habitat, including divergence in diapause timing. Johnson et al. [42] modelled a scenario of this type. An alternative, single-effect, scenario would begin with a proportion of females ovipositing on apple by chance. This favoured alleles for high survival on apple leading to the establishment of a multiple-niche polymorphism. Indirect selection could then have favoured habitat choice through its impact on assortative mating alone, requiring linkage disequilibrium between survival and choice alleles to be established in the face of gene flow and recombination (a form of reinforcement). Distinguishing such alternatives retrospectively is likely to be very difficult.
The importance of phenology in *Rhagoletis* suggests a further option. Apple and hawthorn fruit are temporarily separated habitats. Therefore, any change in diapause timing early in the evolution of the apple race would have constituted a multiple-effect trait under direct selection, because of the benefits of matching timing to the host and also influencing habitat choice. Since the timing difference could have generated assortative mating, this trait would have made three distinct contributions to reproductive isolation.

Interestingly, disrupted host finding in *Rhagoletis* hybrids also contributes to postzygotic isolation [43], a neglected potential contribution of habitat choice to speciation. This constitutes a distinct pathway by which a habitat choice trait can have multiple effects on reproductive isolation, which is independent of the polymorphism versus nonrandom mating criterion. For this reason, we consider the single-effect/multiple-effect distinction separately, because the impact of a habitat choice trait on both polymorphism and nonrandom mating is not the only way in which it can act as a multiple-effect trait.

Another good example of a direct link between habitat choice and adaptation is the pea aphid, *Acyrthosiphon pisum*. In this phytophagous insect, feeding, mating, and oviposition occur only on the host plant. In the northeastern United States, host races of the subspecies *A. pisum pisum* live on alfalfa (*Medicago*) and clover (*Trifolium*), crops that are sometimes grown in adjacent fields, and some gene flow persists between the races [6]. The aphids acceptance of the host plant is one of the main reasons for assortative mating: pea aphids can distinguish between their preferred host and the alternate host by probing with their styles. When they detect the alternate host they do not feed but will move in search of another plant in order to increase their probabilities of reproductive success [44]. Thus, habitat choice and habitat-associated fitness are aspects of the same underlying trait of host acceptance, which can be considered a multiple-effect trait. As with the apple maggot fly, host acceptance also influences assortative mating, and this situation greatly facilitates the evolution of reproductive isolation [2]. It is likely that the key genetic changes of host acceptance involve the aphids chemical senses, and the recent characterisation of chemosensory gene families in the pea aphid [45] opens the way to identification of the responsible genes.

Hawthorne and Via [46] showed that, for the traits they defined as host acceptance and host-associated performance, QTL mapped close together in the pea aphid genome. They suggest that there may be either close linkage between genes for the different traits or alleles with pleiotropic effects on the traits. Following Smadja and Butlin [2], we suggest that it is more instructive to view host acceptance as a multiple-effect trait with direct effects on fitness. Of course, there may be other traits that also adapt the aphids to different host environments, such as mechanisms for tackling host defensive compounds.

### 2.3. “One-Allele” versus “Two-Allele” Mechanisms

“One-allele” and “two-allele” mechanisms have been widely discussed in speciation research since the distinction was introduced by Felsenstein [18] and the distinction has been considered one of the most useful ways to categorise speciation [47]. In principle, habitat choice may evolve by either mechanism [20] but making the distinction for empirical examples is not straightforward. Therefore, we begin with conceptual examples to illustrate the ways in which “one-allele” and “two-allele” mechanisms might apply to habitat choice before suggesting possible case studies.

In “one-allele” mechanisms, a single allele present in a population under divergent or disruptive selection generates habitat preference independently of the direction of selection. One possible way for this to work would be through natal habitat preference induction (reviewed in [48]), in which experience with a natal habitat shapes the preferences of individuals for that habitat. Experience with particular stimuli increases subsequent preference for a habitat that contains those same stimuli, which might help dispersing individuals to locate a suitable habitat quickly and efficiently. Because assessing habitat quality involves time, risk, and energy invested in sampling potential habitats, selection should favour mechanisms that help individuals to select and use habitats that best suit their phenotypes. Any allele that spreads in response to such selection would enhance divergent habitat choice in a population showing local adaptation to multiple niches. Habitat matching, for example in cryptically coloured species, would have a similar effect without the need to condition on the natal habitat (matching habitat choice, [21]), promoting local adaptation and even leading to speciation. On the other hand, in response to environmental change, adaptation could involve change or relaxation of habitat choice instead of adaptation to modified conditions, resulting in more variable habitat use [21], which could lead to breakdown of reproductive isolation. Under these conditions the “two-allele” mechanism would offer greater resistance to hybridisation than the “one-allele” mechanism, although “two-allele” habitat choice would be less likely to initiate the process of speciation.

“Two-allele” habitat choice is more likely to involve innate preferences for specific habitat features, such as substrate colour or odour or the presence of particular resources. Divergent or disruptive selection is required to establish such distinct preferences.

Differentiating between “one-allele” (e.g., habitat matching) and “two-allele” (distinct preferences in different sub-populations) scenarios is not an easy task, as previously mentioned. As an example, we consider colour morphs of another phytophagous insect, the walking stick *Timema cristinae* (see [49] for a review). Two host species with highly divergent leaf colour and shape, *Ceanothus spinosus* and *Adenostoma fasciculatum*, are utilised. Insects found on different hosts have different cryptic colour patterns because of selection due to predation. These wingless insects feed, mate, and reproduce on the same host individual and movement between plants is restricted (12 m per generation, [50]). They show host preference [51] and partial assortative mating [52]. Immigrant inviability (selection against ecotypes from the contrasting habitat; i.e., host plant) is also an important process maintaining the ecotype divergence [53]. It is clear that habitat choice contributes to the maintenance of *Timema* ecotypes but the mechanistic basis of this
preference has not yet been characterised. The preference could, for example, involve detection of different chemical compounds on the host plant surfaces that are unrelated to crypsis: a “two-allele” mechanism. Alternatively, the insects may match their own colouration to the background, a mechanism that could fall into the “one-allele” category. These possibilities could be distinguished experimentally, for example by allowing insects to choose between backgrounds of different colour/pattern in the absence of plant material.

Preferences for divergent chemical signals are likely to underlie host-plant preference in many phytophagous insects [54]. These are likely to involve “two-allele” mechanisms where different alleles for positive or negative responses [55] to particular stimuli have to be fixed in the diverging subpopulations and have to be associated, through linkage disequilibrium or pleiotropy, with traits that underlie local adaptation. Because this is less favourable to speciation than matching mechanisms, it is important to make the distinction in more case studies.

Particularly interesting possible cases of “one-allele” habitat choice involve learning, including imprinting. Such cases are sometimes described as “nongenetic” but clearly the ability to imprint or the strength of imprinting can have a genetic basis and the spread of a single allele can then cause divergent habitat preferences by promoting imprinting on different signals. Obligate brood parasitic birds represent potential examples of this process. In the continuum of divergence and/or speciation [4, 56, 57], different races or species pairs represent different stages in the process of ecological speciation, and this is also the case for brood parasitic birds. Indigobirds (genus Vidua) are obligate, host-specific brood parasites of firefinches (genus Lagonosticta) and other estrildid finches (family Estrildidae). Assortative mating is due to song learning and mimicry of the host song by the males [58, 59], and this allows a host switch to occur in a single generation. Females also learn the song of their foster parents and choose their mates and the nests they parasitise using song. Different degrees of divergence are found depending on the species under study, for example, some of them are morphologically indistinguishable [60, 61] and they lack genetic differentiation at neutral markers [62]. When host species of different indigobirds have overlapping distributions, hybridisation can occur due to egg-laying mistakes (e.g., a female lays an egg in a nest parasitised by another indigobird species). Individuals of different species would then learn the same song, through imprinting, and are likely to hybridise. However, in most of the cases, indigobird species have evolved several other polymorphisms, such as different male plumage colour and nestling mouth markings that match those of their respective hosts [63], thereby enabling young indigobirds to better compete for parental care in host nests. These polymorphisms represent different axes of divergence (greater “ecological niche dimensionality,” [64]) promoting increased divergence, despite the possibility of accidental hybridisation in regions where host ranges overlap. This suggests that, following colonisation of a new host, host fidelity due to imprinting can be sufficient for divergent natural and/or sexual selection on morphology, ecology, and/or behaviour to generate progress on the speciation continuum. Imprinting causes habitat choice, through its influence on female choice of host nests, as well as mate choice and so is a multiple-effect as well as a “one-allele” trait [2, 7].

In these sections, we have selected empirical examples that illustrate each of our classifying criteria. Figure 1 shows the ways in which these criteria may combine to create conceptual scenarios with varying probabilities of progression to speciation. Working from the framework, how easily can these conceptual scenarios be applied to real-world systems? Our brief review of the literature suggests that habitat choice studies pertaining to ecological speciation are biased towards phytophagous insects. This is not surprising, because “host race” formation seems to be a common route to speciation, which has been widely studied. However, in an attempt to expand the scope of habitat choice studies in an ecological speciation context, we discuss below the evidence for habitat choice in the intertidal gastropod genus Littorina. We examine the current evidence for habitat choice, discussing which scenarios are likely to be involved and the difficulties in trying to distinguish them.

3. Habitat Choice in Littorina

Intertidal gastropods present ideal systems for studies of habitat choice: the littoral zone can create extreme environmental gradients and highly heterogeneous habitats within relatively short distances, and generally the animals are easy to locate and manipulate for both in situ and lab-based trials. Heterogeneous habitats of this type can lead to differential survival and generate divergent selection in polymorphic populations. Microhabitat use in this landscape has been identified as strongly influencing survival in intertidal gastropods [65–67], so habitat choice presents itself as a likely trait to respond to this selection.

Large-scale transplant experiments have indicated habitat preference behaviour in Littorina species, such as L. keena [68], L. angulifera [69], and L. unifasciata [70]. All show that the snails tend to return to the approximate tidal height from which they were displaced, exhibiting directional movement towards the shore level of origin. However, these transplant experiments may also be influenced by effects of differential survival that are hard to separate from behavioural effects. We will examine this problem below.

Littorina saxatilis, the rough periwinkle, is a marine gastropod that is emerging as a model system for studying ecological speciation. It is widely distributed across rocky shorelines in the North Atlantic, extremely polymorphic (shell colour, shell shape, and behaviour), and prone to ecotype formation due to local adaptation because of its low average dispersal [71]. Pairs of phenotypically divergent ecotypes occupying different niches in the intertidal zone are found over scales of tens of metres or shorter across different shores along its distribution and are maintained through divergent natural selection [72]. These ecotypes of L. saxatilis have been studied in detail on shores from three geographical regions (Sweden, UK, and Spain), and a process of parallel ecological speciation between them has been suggested ([72], but see [73, 74]). However, despite displaying phenotypic
The ecotype pairs on each of these shores are separated on a microgeographic scale, exhibiting adaptations to the prevailing habitat. On Swedish shores, the habitat is composed of a mosaic of cliff habitat punctuated with boulder fields, whereas the UK and Spanish ecotype pairs are found on the same shores, but at different levels of the littoral zone. The ecotypes in the UK are known as H and M (high-shore and midshore), those in Sweden are termed E and S (exposed and sheltered), and the Spanish pair are termed RB and SU (ridged-banded and smooth-unbanded) (see [73]). The M, S, and RB ecotypes are morphologically congruent, exhibiting thick shells, relatively small shell apertures, and large body size. These features are considered to be adaptations to an important selection pressure: predation by crabs. The H, E, and SU ecotypes from these three shores also share similar morphological characters: smaller size, thinner shells, and a larger shell aperture. In avoiding the hazards of crab predation by their position on the shore (low in Spain, high in Britain and Sweden), these ecotypes are free to develop larger shell apertures, increasing foot area and thus grip on the substrate to minimise dislodgement. Nonrandom mating is also observed in each population of ecotype pairs [78–81], primarily due to assortative mating by size (see [72] for a review). L. saxatilis lacks a pelagic larval stage, instead exhibiting direct development where females retain their brood internally and release fully formed young [71]. Many other littorinid species (such as L. littorea) produce a pelagic larval stage, allowing dispersal over a wide range and maintaining gene flow between populations [71]. The low dispersal range of L. saxatilis (1–4 m, [82, 83]) limits gene flow, and this facilitates much greater local adaptation in this species than in many of its congeners [72].

Since selection drives differential adaptation to closely adjacent habitats, habitat choice mechanisms could easily be imagined to play a role in population divergence. Random dispersal combined with selection against less fit phenotypes may superficially look like habitat choice as the phenotypes are segregated into divergent habitats, as noted above. This is particularly true where dispersal distance is short allowing selection to produce sharp phenotypic transitions at habitat boundaries. Selection for reduced postnatal dispersal [84] may accentuate this effect. However, as there is no active behavioural mechanism, this does not constitute habitat choice as we define it.

A possible exception, where habitat choice can be inferred from phenotypic distributions, is where habitat heterogeneity is on a scale much smaller than the dispersal distance. It is then not possible for selection to maintain genetic differentiation between patches [85] independent from habitat choice, although phenotypic plasticity could still result in strong phenotype-habitat associations. Morphological and AFLP (amplified fragment length polymorphism, see [86] for a review) clines have been identified, which are too steep to have been generated by selection alone [87, 88]. In these cases, additional mechanisms such as habitat choice may contribute to the genesis and maintenance of the gradient by strengthening barriers to gene flow. In the middle of the shore gradient in Spain, mussel and barnacle dominated patches are intermingled on a scale of a few centimetres and the RB and SU L. saxatilis ecotypes are associated with these patches [89]. This is strongly suggestive of active habitat choice [90]. The heterogeneous nature of this connecting habitat may be particularly important in the maintenance of the hybrid zone and the segregation of the ecotypes, as has been demonstrated with Bombina toads [91]. Nevertheless, in order to determine the role of habitat choice in maintaining divergent populations, in L. saxatilis and in other comparable systems, it is necessary to utilise manipulative experiments (e.g., using mark-recapture approaches).

Clear evidence for home-site advantages in littorinid species has been documented [83, 89, 92] along with evidence of selection on shell characters. In this context, we consider a home-site advantage to be where individuals are likely to have increased fitness in the habitat or microhabitat to which their ecotype may be presumed to be adapted. This advantage may vary at different stages of the life history. Is there also good evidence for habitat choice in the L. saxatilis ecotypes? Has L. saxatilis evolved habitat choice in response to divergent selection, or did nonrandom mating and adaptive polymorphism evolve in the presence of preexisting habitat choice? Habitat choice can be an adaptive behaviour, increasing fitness in the “home” habitat even when only a single habitat type is occupied [93] and so could have been present before ecotype differentiation began. Is habitat choice a multiple-effect trait in Littorina and is it based on “one-allele” or “two-allele” genetic variation? We discuss the evidence for the presence of habitat choice in L. saxatilis and consider whether it is possible to make any of these distinctions.

Work on Swedish populations indicates that morphological adaptation to the contrasting environment has a strong genetic basis but has an element of plasticity which can improve local adaptation [94, 95]. However, the E (exposed) ecotype displayed significantly lower levels of plasticity than the S (sheltered) ecotype, indicating differential plasticity within local populations. This leads to the question of the effect of plasticity on the role of habitat choice: is it feasible that lower phenotypic plasticity might favour the evolution of genetically based habitat choice, to increase occupation of the environment in which individuals are more fit. Increased plasticity may decrease selection for habitat choice, since individuals would be better able to adapt their phenotype to local conditions. This might be tested if the degree of morphological plasticity varies among regions, leading to a prediction of varying habitat choice.

Experimental evidence for nonrandom dispersal in L. saxatilis ecotype populations has been obtained in both Spain and Sweden [78, 82, 83]. In the Swedish populations, displaced snails exhibited greater average dispersal distances than nondisplaced ones and dispersal differed between E and S ecotypes, in addition to a tendency to recapture snails in their own habitat more often than expected from random dispersal [83]. However, this tendency to recapture snails in their own habitat may be a function of differential survival
in native and nonnative habitats. Additionally, although survival rates and migration distances were measured, direction of movement was not. Erlandsson et al. [82] expanded on this study to determine whether the dispersal was directional in the Spanish population. They detected random dispersal when snails were placed at their native shore level (with overall dispersal distances averaging less than 2 m), whereas when animals were transplanted to their nonnative shore level they moved further and more directionally, with the Spanish RB morph exhibiting the greatest directional response. Although this hinted at habitat choice in this ecotype, the recapture rate was low (<20%) and conclusions were drawn only from recaptured individuals (which are likely to be a strongly selected sample), therefore it is difficult to make any meaningful conclusions about habitat choice from these simpler experimental studies.

On the Galician shore in Spain, Cruz et al. [78] tried to separate survival and habitat preference in the two ecotypes of \textit{L. saxatilis} using two reciprocal transplant experiments. In the first experiment, sample groups of each ecotype were transplanted both to their native and nonnative shore level at each of two sites. Snails were then recaptured and their movement recorded from two days after transplant. The study compared the recapture positions of the transplanted snails and the recapture positions of the corresponding control snails, to correct for movements that may have been induced via prevailing climatic conditions. In addition to the confounding effect that such forced migration might have had on the snails survival, transplanted snails may have dispersed beyond the study area leading to reduced recapture. One way in which Cruz et al. [78] avoided these complications was to argue that only directional movement could result in more than 50% of the released snails in the treatment group being recaptured in the direction of their preferred habitat (up shore for RB, down shore for SU). With this stringent criterion, habitat choice was only observed in one site.

The second experiment involved collection of snails from five intertidal levels on each of two sites and reciprocal transplants across sites for release. This destroyed the correlation between shore level and snail phenotype (measured as the first axis of shell shape variation). Over a period of two weeks, they observed the reformation of the shell-shape cline and measured the relative contributions, for the cline reformation, of the snails migrating movements and survival. Using a clever comparison between the change in average positions of all snails recaptured and the change in position of those that were known to have survived, they separated the contributions of differential survival and habitat choice to the changing cline. Differential movement contributed between one-third and one-half of the change in cline at one site and hardly at all at the other site, leading to the conclusion that habitat choice was present but less important than differential viability in the maintenance of the phenotypic cline.

This important study illustrates the difficulties associated with demonstrating habitat choice in the field. Despite considerable effort and thoughtful design, the experiments were still hampered by low recapture rates (around 50%) and could be criticised for releasing snails at high densities in potentially unnatural positions. The analyses do not provide quantitative estimates of survival, dispersal, or their ecotype-habitat interactions, the sort of variables that would be needed to model likely evolutionary scenarios. A recent theoretical model of ecotype formation in \textit{Littorina saxatilis} [96] did not include habitat choice as a parameter. Due to the currently unknown contribution of habitat choice to reproductive isolation in this species, it would be interesting to see how the potential to evolve choice might influence model outcomes.

Cruz et al. [78] also discussed the possible behavioural basis of habitat choice. The observation that shell morphology provided the best predictor of habitat-specific viability but that sampling location best explained the pattern of movement led them to suggest that shell shape and habitat choice are genetically independent. Therefore, in the terms we use here, shell shape is not a multiple-effect trait in the sense that a change in shape alters the fitness profile but does not automatically alter habitat preference (as it might if snails had a pre-existing tendency to move to a habitat that was favourable for their shell shape). Note, however, that shell size does seem to be multiple-effect in that it influences both differential survival and assortative mating. A “two-allele” mechanism (or “double-variation”; [20, 78]) seems more likely than a “one-allele” mechanism for the same reason. Movement to the optimum habitat could be a “one-allele” mechanism but would result in a strong association between shell shape and differential movement, which may occur prior to local adaptation. An upward bias in RB and a downward bias in SU would most likely be a two-allele mechanism, dependent on linkage disequilibrium and so less tightly linked to shape. Under this assumption, habitat choice could evolve after local adaptation. Lack of preference in hybrids [90] tends to support this conclusion, suggesting that habitat choice evolved after local adaptation whereas a tendency toward matching position to optimal habitat preceded local adaptation. However, direct behavioural tests and genetic analysis are needed to confirm these speculations and will be difficult if habitat choice is as weak in other regions as it seems to be in Spain.

Other littorinid studies highlight the role of chemoreception in influencing the behaviour of individuals (such as trail following: [97, 98]). It has been determined that \textit{L. saxatilis} E ecotype males (S males were not studied) are able to discriminate between mucous trails of the female of each ecotype [99] and show a clear preference for trails of females within the size range of the E ecotype females. In addition to its role in assortative mating, trail following could play a role in habitat choice. This has been studied to an extent in \textit{L. littorea} [100]. When chemical cues were removed from the “home” boulder and substrate, \textit{L. littorea} displayed a significantly impaired ability to navigate back to the boulder from which they had been displaced. In \textit{L. saxatilis} it would be interesting to separate the role of assortative mating from habitat choice. Trail following could impact dispersal experiments by making individual movements nonindependent [78] and, if it forms the basis of philopatry, could represent a “one-allele” habitat choice.
mechanism. More studies are needed to unveil the role of chemoreception in habitat choice in this species.

Did size-assortative mating evolve after ecological partitioning and evolution of habitat choice? Or did habitat choice facilitate ecotype formation after the development of assortative mating? The model by Sadedin et al. [96] suggested that assortative mating may be considered ancestral. However, although a number of ecological factors were modelled, habitat choice was not included as a parameter. Dispersal was included, but this was not directional. Dispersal was an important consideration though: frequent long-range random dispersal eliminated spatial genetic structure and did not lead to ecotype formation. Although the role and mechanism of habitat choice in *Littorina* have not yet been explicitly modelled, we may draw inferences from models developed for other organisms. Early models suggested that when fitness, mating, and habitat choice are all based on the same character, speciation with gene flow may result—the degree of reproductive isolation is determined by the strength of assortative mating and the strength of disruptive selection. For moderate selection, habitat-based nonrandom mating also facilitates reproductive isolation. However, in simulations, the size-related mate choice mechanism in *L. saxatilis* could not explain more than a small part of the sexual isolation between morphs [101]. This implies that size-related mate choice, although considered a multiple-effect trait, may only be important in a speciation context if it evolves in parallel with other ecological traits, including habitat choice.

These studies highlight the difficulties in connecting theoretical evolutionary scenarios with existing empirical data. However, if future habitat choice studies are carried out with an explicit conceptual framework in mind and across a wide range of study systems, they will contribute more effectively to our understanding of speciation.

### 4. Conclusion

The influence of habitat choice on ecological speciation clearly varies in both magnitude and mechanism, and in many cases we cannot be sure about the contribution it makes to reproductive isolation or at what stage it evolved. The empirical examples discussed for some of our projected scenarios provide an indication of which evolutionary scenarios have been observed in natural systems. We would expect those scenarios where habitat choice does not strongly favour progress towards speciation to be detected in studies of within-species polymorphism, whereas those promoting speciation may be more prevalent among studies of ecological speciation. A more exhaustive review is needed to test this prediction but may be premature since many case studies do not yet provide enough information to distinguish among scenarios for the evolution of habitat choice.

Although there have been some valuable habitat choice studies on *Littorina saxatilis*, there are still a lot of unanswered questions regarding its role in the maintenance of both the phenotypic and genetic clines. As a candidate system for ecological speciation, the understanding of the role of habitat choice prior to complete reproductive isolation in *L. saxatilis* is an important facet in our overall understanding of the processes and mechanisms leading to species formation.

Describing the role of habitat choice within the conceptual framework that we propose represents an important step in understanding speciation. It shows how habitat choice can affect reproductive isolation in very different manners, influencing the likelihood of speciation and potentially leading to different stages along the continuum of speciation. Empirical studies of habitat choice in divergent populations or closely related species, representing different stages of speciation and different evolutionary scenarios, should form a focus for future research. When analysed within such a conceptual framework, we believe these studies will give more insight into the part that habitat choice plays in ecological speciation than if they are considered in isolation.

### Acknowledgments

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### References


Research Article

The Role of Environmental Heterogeneity in Maintaining Reproductive Isolation between Hybridizing Passerina (Aves: Cardinalidae) Buntings

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Hybrid zones are useful systems in which to investigate processes important in creating and maintaining biological diversity. As they are often located in ecotones, patterns of environmental heterogeneity may influence hybridization, and may also influence the maintenance of reproductive isolation between hybridizing species. Focusing on the hybrid zone between Passerina amoena (Lazuli Bunting) and Passerina cyanea (Indigo Bunting), located in the eastern Rocky Mountain/western Great Plains ecotone, we examined the relationship between population-pairwise differences in the proportion of hybrids and environmental variation. Models including environmental variables explained more of the variation in hybridization rates across the ecotone than did models that only included the geographic distance between sampling localities as predictor variables (63.9% and 58.9% versus 38.8% and 39.9%, depending on how hybridization was quantified). In the models including environmental variables, the amount of rainfall during the warmest quarter had the greatest explanatory power, consistent with a hypothesis that P. cyanea is better adapted to the mesic environments of eastern North America and P. amoena is better adapted to the xeric habitats of western North America. These results suggest that continued reproductive isolation between these species is mediated, at least partially, by differential adaptations to local environmental conditions.

1. Introduction

Hybrid zones formed through the interbreeding of divergent lineages as a result of secondary contact are powerful models for investigating the forces important in generating and maintaining biodiversity [1–5]. These “natural laboratories” [1] provide an opportunity to explore patterns of gene flow and introgression between closely related taxa, which can aid in the identification of genes contributing to reproductive isolation [6, 7]. In addition, naturally occurring hybrid zones allow the investigation of how and to what extent ecological differences between the hybridizing lineages contribute to speciation [8, 9].

Divergent natural selection between different habitats can be an important force in promoting speciation through its potential to cause habitat isolation between closely related taxa [10–12]. When traits subject to divergent natural selection are associated or correlated with traits that may influence reproduction, ecological speciation may result [12]. Naturally occurring hybrid zones are often located in ecotones produced by sharp transitions between different environments in which the hybridizing species were subjected to divergent natural selection [13, 14]. Such ecotones are often characterized by areas of marginal habitat that may not be ideal for the parental species found on either side of the environmental gradient and therefore provide an opportunity to examine the impact of habitat on hybridization and the evolution of reproductive isolation [8, 11]. In this manner, hybrid zones offer a chance to explore the environmental conditions that were potentially
important to the initial divergence of the hybridizing species, providing insight into the importance of ecological speciation, mediated through divergent natural selection.

Spatial environmental heterogeneity can change over time, with the potential to greatly alter the structure of hybrid zones, and influence speciation and biodiversity dynamics [8]. If hybridizing species are differentially adapted to the environments they primarily inhabit, changes in those environments and the intervening ecotone may result in one of two general outcomes: (1) reduction in species diversity—one species may swamp the other, causing extinction of the overwhelmed species, or the species may collapse into a hybrid swarm wherein both parental species in essence become extinct, but a new “hybrid” species is formed—or (2) reduction or cessation of hybridization—if hybridization only occurs in the “hybrid” habitat found in an ecotone and such habitats are altered or eliminated. Alternatively, environmental stability may promote the stability of a hybrid zone, regardless of whether the zone is maintained through endogenous selection against hybrids balanced by continued migration into the zone (i.e., a tension zone) or exogenous selection creating fitness differences along the environmental gradient found in the ecotone.

In this paper, we quantify the relationship between patterns of environmental heterogeneity and patterns of hybridization across an ecotone, using the hybrid zone formed between Lazuli (Passerina amoena) and Indigo (Passerina cyanea) buntings as a model. We explore whether differences between populations in the proportion of hybrid individuals are better predicted by the variation found along the ecological gradient experienced across the hybrid zone than by simple geographic distance between populations. In doing so, we investigate the potential role of ecological differences between P. amoena and P. cyanea in maintaining reproductive isolation between these species.

Passerina amoena and P. cyanea are closely related passerines [15] that form a hybrid zone in the ecotone located between the eastern Rocky Mountains and the western Great Plains of North America (Figure 1; [16–18]). Behavioral studies, carried out in both the lab and the field, demonstrate strong patterns of assortative mating and show that, when choosing mates, females of both species place greater emphasis on male plumage patterns than on song characteristics [19–21]. Field-based research also suggests that hybrid individuals suffer fitness consequences relative to pure individuals. Mated pairs that involve at least one hybrid individual produce fewer nestlings and fledglings than do pairs involving only nonhybrid individuals [21].

More recently, coalescent analyses of the evolutionary history of P. amoena and P. cyanea suggest that speciation occurred through either parapatric divergence or repeated cycles of allopatric divergence with gene flow during periods of secondary contact [22]. Regardless of the spatial context of divergence, the current hybrid zone originated through secondary contact and is probably no older than ~6500 years [7, 22, 23]. Patterns of introgression across the hybrid zone, based on allelic data from two mitochondrial genes, four nuclear autosomal loci, and two sex-linked loci, indicate reduced introgression of mitochondrial DNA and sex-linked loci, relative to the pattern seen for nuclear autosomal loci [23], a finding consistent with Haldane’s rule [24].

Additional work has shown that introgression patterns of sex-linked loci can vary substantially [7]; maximum likelihood estimates of 10 locus-specific cline widths differed by ~200-fold, identifying a candidate genomic region for reproductive isolation. Suggestively, the sex-linked locus with the narrowest cline width is an intron in the very-low-density lipoprotein receptor gene (VLDLR); mutations in this gene are known to reduce egg laying capabilities in chickens [25] and it is possible that divergent P. cyanea and P. amoena VLDLR alleles do not function well in a heterospecific genomic background, which could cause hybrid females to lay fewer eggs. The hypothesis that a Dobzhansky-Muller incompatibility [26–28] causes a reduction in egg laying in hybrid females is consistent with the field research findings of Baker and Boylan [21] described above.

Using a species distribution modeling approach, Swenson [29] suggested that the ecotonal environment between the eastern Rocky Mountains and western Great Plains is important in determining the geographic location of the Passerina bunting hybrid zone, with temperature differences having the biggest impact. This may partially explain the observed shift in location and width of the hybrid zone that has occurred over the past 40–45 years [30]. However, Swenson’s [29] analysis utilized only the geographic location of hybrid individuals; here we quantify the proportion of hybrid individuals within a population to elucidate the influence of environmental heterogeneity on hybridization dynamics.

2. Materials and Methods

2.1. Genetic Data and Analyses. Population samples of P. amoena, P. cyanea, and P. amoena × P. cyanea hybrids were collected from 21 sympatric and parapatric localities across the contact zone during May, June, and July 2004–2007 (Figure 1, Table 1). All collected individuals were prepared as voucher specimens and deposited in the Louisiana State University Museum of Vertebrates. Additional samples from allopatric populations (P. amoena: WA, ID; P. cyanea: MN, IL, MI) were also obtained (Figure 1, Table 1).

From each individual, genomic DNA was extracted from pectoral muscle and used to generate sequence data from a suite of 14 loci (see Supplementary Table in Supplementary Material available online at doi: 10.1155/2011/295463 Table S1) as previously described [7, 22]. We resolved individual haplotypes probabilistically using PHASE [33, 34] and then identified the largest independently segregating block of sequence for all loci as in Carling and Brumfield [7, 22]. Individual haplotypes with frequencies ≥0.80 in the “allopatric” P. amoena population (WA) were classified as P. amoena haplotypes, and haplotypes with frequencies ≥0.80 in the “allopatric” P. cyanea populations (MN, IL, MI) were designated as P. cyanea haplotypes [7, 22]. We then generated multilocus genotypes for each individual by coding each allele at each locus as either P. amoena or P. cyanea.
Using these multilocus genotypes we quantified the genetic ancestry of each individual using STRUCTURE [35–38]. In the analyses, the allopatric populations (P. amoena: WA, ID; P. cyanea: MN, IL, MI) were coded as “learning populations” (USEPOPINFO model). For all other individuals, we estimated the proportion of their ancestry attributed to the two clusters defined by the “learning populations.” We used the default allele frequency model and ran the analysis three times, each time with a burnin length of 100,000 steps followed by a postburnin run of 1,000,000 steps. We then calculated the mean ancestry values across the three runs for each individual from the sympatic and parapatric populations. Individuals were classified as a “hybrid” according to two different criteria: (1) “80–20” wherein an individual was classified as a hybrid if the proportion of its genetic ancestry assigned to cluster 1 (P. amoena) was between 0.80 and 0.20 and (2) “90–10” wherein an individual was classified as a hybrid if the proportion of its genetic ancestry assigned to cluster 1 was between 0.90 and 0.10. In both scenarios, individuals whose proportion of genetic ancestry assigned to cluster 1 was greater than 0.80 (criterion 1) or 0.90 (criterion 2) were classified as pure P. amoena individuals and all other individuals were classified as pure P. cyanea individuals.

Within each sympatic and parapatric population, we calculated the proportion of hybrid individuals, which were then used to generate a distance matrix of the relative similarity in hybridization among populations. The “hybridization distance” between two populations was calculated as:

$$\frac{|(PL_1 + 0.5PH_1) - (PL_2 + 0.5PH_2)|}{2}$$

where PL_1 is the proportion of P. amoena individuals in population 1, PH_1 is the proportion of hybrids in population 1, PL_2 is the proportion of P. amoena individuals in population 2, and PH_2 is the proportion of hybrids in population 2. This hybridization distance was calculated for every pair of populations, except the allopatric ones (WA, ID, MN, IL, MI) and the resultant matrix (Table 2) formed...
Table 1: Sampling localities, sample sizes, and the proportion of hybrid individuals for populations analyzed in this study.

<table>
<thead>
<tr>
<th>Code</th>
<th>Locality</th>
<th>Latitude</th>
<th>Longitude</th>
<th>N</th>
<th>Proportion of hybrids (80–20)</th>
<th>Proportion of hybrids (90–10)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>WA</td>
<td>47.8</td>
<td>−118.9</td>
<td>29</td>
<td>—</td>
<td>—</td>
</tr>
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<td>2</td>
<td>ID</td>
<td>44.1</td>
<td>−116.2</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>MT: Custer National Forest no. 1</td>
<td>45.1</td>
<td>−108.5</td>
<td>4</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>WY: Bighorn National Forest no. 1</td>
<td>44.8</td>
<td>−107.3</td>
<td>8</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>WY: Bighorn National Forest no. 2</td>
<td>44.6</td>
<td>−107.7</td>
<td>2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>CO: Roosevelt National Forest</td>
<td>40.7</td>
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<td>7</td>
<td>0.14</td>
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</tr>
<tr>
<td>7</td>
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<td>−106</td>
<td>5</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>8</td>
<td>WY: Medicine Bow National Forest</td>
<td>42.4</td>
<td>−105.3</td>
<td>3</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td>ND: Little Missouri National Grassland</td>
<td>46.8</td>
<td>−103.5</td>
<td>13</td>
<td>0.00</td>
<td>0.00</td>
</tr>
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<td>10</td>
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<td>10</td>
<td>0.00</td>
<td>0.00</td>
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<td>11</td>
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<td>44.5</td>
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<td>14</td>
<td>0.36</td>
<td>0.50</td>
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<tr>
<td>12</td>
<td>NE: White River*</td>
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<td>−103.5</td>
<td>8</td>
<td>0.38</td>
<td>0.50</td>
</tr>
<tr>
<td>13</td>
<td>SD: Black Hills National Forest</td>
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<td>−103.8</td>
<td>5</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>14</td>
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<td>7</td>
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<td>8</td>
<td>0.25</td>
<td>1.00</td>
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<td>4</td>
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<td>0.50</td>
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<tr>
<td>17</td>
<td>NE: Nenzel*</td>
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<td>−101.1</td>
<td>4</td>
<td>0.25</td>
<td>0.25</td>
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<tr>
<td>18</td>
<td>NE: The Nature Conservancy</td>
<td>42.8</td>
<td>−100</td>
<td>18</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>19</td>
<td>SD: Carpenter Game Production Area</td>
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<td>−99.5</td>
<td>13</td>
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<td>0.08</td>
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<td>20</td>
<td>ND: The Nature Conservancy Pigeon Point Preserve</td>
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<td>−97.3</td>
<td>4</td>
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<td>10</td>
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<td>0.00</td>
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<td>SD: Newton Hills Game Production Area</td>
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<tr>
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<td>MN</td>
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<td>—</td>
</tr>
<tr>
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<td>IL</td>
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<td>−88.9</td>
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<td>—</td>
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</tr>
<tr>
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<td>MI</td>
<td>42.3</td>
<td>−83.7</td>
<td>7</td>
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* Access to these localities generously provided by private landowners.

the basis of our generalized dissimilarity modeling (see below). As an alternative measure of “hybridization distance” between two populations, we also calculated the absolute difference between mean population Q-scores, as estimated by STRUCTURE [35–38].

2.2. Spatial Data and Analyses

2.2.1. Environmental Data. We used a set of moderately high-resolution climate and satellite remote sensing variables to characterize the habitat within and on both sides of the hybrid zone (Table 3). Climate data were obtained from the WorldClim database [39], which are spatially explicit estimates of annual means, seasonal extremes, and degrees of seasonality in temperature and precipitation based on a 50-year climatology (1950–2000). The climate variables that were included in our analyses are *annual mean temperature* (Bio1); *mean diurnal temperature range* (Bio2), which is the difference in daily maximum and minimum temperatures, averaged across the year; *mean temperature of the warmest quarter* (Bio10); *mean temperature of the coldest quarter* (Bio11); *total annual precipitation* (Bio12); *precipitation seasonality* (Bio15), which is the coefficient of variation in monthly rainfall across the year; *total precipitation of the wettest quarter* (Bio17); *total precipitation of the warmest quarter* (Bio18).

In addition to these ground-based measurements of climate, we used satellite remote sensing data from both passive optical sensors (MODIS; https://lpdaac.usgs.gov/lpdaac/products/modis_overview) and active radar scatterometers (QuickScat; http://www.scp.byu.edu/data/Quikscat/SIRv2/qush/World_regions.htm) to infer a variety of ecological characteristics of the land surface. From the MODIS archive, we used the average monthly Normalized Difference Vegetation Index (NDVI) to infer vegetation density [40]. NDVI is sometimes also referred to as a measure of
Table 2: Matrix of hybridization distances \(|(PL_1 + 0.5PH_1) - (PL_2 + 0.5PH_2)|\); see text for details) between all pairs of sympatric and allopatric populations. Above the diagonal, hybrids were identified using the "80–20" criterion; below the diagonal, hybrids were identified using the "90–10" criterion.

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“greenness” and is high over forested areas and low over areas with sparse vegetation. In addition, we used the vegetation continuous field [41] product as a measure of the percentage of tree cover. In contrast to NDVI, percent tree cover specifically measures the land surface area covered by trees, disregarding other types of vegetation. From QuickScat (QSCAT), we obtained raw backscatter measurements for the month of August that capture attributes related to surface moisture [40], and, from the Shuttle Radar Topography Mission (SRTM), we acquired elevation data.

Time series of the remote sensing data sources were acquired from 2001 for QSCAT and tree cover and means over the period 2000–2004 for NDVI. Variables with native resolutions higher (e.g., SRTM: 30 m) or lower (e.g., QSCAT: 2.25 km) than 1 km were reaggregated to a 1 km grid cell resolution. This resolution is often used in ecological niche modeling at the regional or subcontinental scales and balances resolutions from climate data, which often have coarser native resolutions, and remote sensing data, with higher native resolutions. To improve interpretation, we checked for covariance among variables and only included those with substantial unique variance. Various criteria were used to decide which layers of correlated pairs (with Pearson’s correlations on the order of 0.9 or larger) were retained for further analysis. These included keeping layers that are more commonly used in distribution modeling (WorldClim) or that exhibit larger contrast/variance over the study area (QSCAT) as well as having best data quality (NDVI). Pearson’s cross correlations of the used environmental variables are shown in supplementary Table S2.

2.2.2. Generalized Dissimilarity Modeling. To analyze the relative contribution of geographic distance and environmental variables to explaining the similarity among sampling locations in the percentage of hybrids, we used generalized dissimilarity modeling (GDM, [42]). GDM is a matrix regression technique that predicts biotic dissimilarity (turnover) between sites based upon environmental heterogeneity and geographic distance. One advantage of GDM over other modeling methodologies is that it can fit nonlinear relationships between predictor and response variables through the use of I-spline basis functions [42]. Another advantage of GDM is that, because it uses pairwise comparisons between sites, it can explicitly take into account the influence of geographic distance on explaining biological variation—a particularly important feature in our study. The relative importance of predictor variables in a GDM can be assessed by means of response curves. To further evaluate the extent to which geographic distance is potentially correlated with environmental differences, we ran independent tests with the following sets of predictor variables: (1) environmental variables and geographic distance; (2) only geographic distance; (3) only environmental variables.

GDM is a two-step method: first, dissimilarities of a set of predictor variables are fitted to the genetic dissimilarities (the response variables). The contributions of predictor variables to explaining the observed response variation are tested by permutations, and only those variables that are significant are retained in the final model. These procedures result in a function that describes the relationship between environmental and response variables. Second, using the function resulting from the first step, a spatial prediction is made of the response variable patterns. For visualization purposes, classes of similar response are color coded, where larger color differences between two localities represent larger differences in the proportion of hybrid individuals. In order to assess the significance of the level of variation that was explained by our models, we ran additional models in which the environmental layers were substituted by layers with random values for each grid cell. The resulting percentage of variation explained was compared to that of the full model. We considered the performance of the full model not significant if it explained an equal amount or less of the total variation than a model with random environmental variables.

<table>
<thead>
<tr>
<th>Data Record</th>
<th>Instrument</th>
<th>Ecological attributes</th>
<th>Variables derived</th>
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<td>Satellite-MODIS</td>
<td>Vegetation density</td>
<td>NDVI mean</td>
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<td>Vegetation Continuous Field (VCF)(^*)</td>
<td>Satellite-MODIS</td>
<td>Forest cover + heterogeneity</td>
<td>Tree cover</td>
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<td>Scatterometer-Backscatter(^*)^(^1)</td>
<td>Satellite-QSCAT</td>
<td>Surface moisture + roughness (forest structure), seasonality</td>
<td>QSCAT aug</td>
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<td>DEM(^*)</td>
<td>SpaceShuttle SRTM</td>
<td>Topography + ruggedness</td>
<td>SRTM</td>
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<td>WorldClim(^*)</td>
<td>Station-Network</td>
<td>Bioclimatic variables</td>
<td>Bio1, Bio2, Bio10, Bio11, Bio12, Bio15, Bio17, Bio18</td>
</tr>
</tbody>
</table>

*Data at native resolutions smaller or larger than 1 km have been aggregated to 1 km.
\(^1\)QSCAT is based on data from 2001.
\(^*\)Based on MODIS data from 2001 [41].
\(^\$\)WorldClim data are based on monthly climatologies from 1950–2000 [39]. The bioclimatic variables are annual mean temperature (Bio1), mean diurnal temperature range (Bio2), mean temperature of the warmest quarter (Bio10), mean temperature of the coldest quarter (Bio11), annual precipitation (Bio12), precipitation seasonality (Bio15), precipitation of the wettest quarter (Bio17), and precipitation of the warmest quarter (Bio18).
3. Results

3.1. Genetic Analyses. Using multilocus genotypes, the proportion of hybrid individuals in the 21 sympatric and parapatric populations ranged from 0.00 to 0.57 using the “80–20” criterion and 0.00 to 1.00 using the “90–10” criterion (Table 1). Across all sympatric and parapatric populations, 20 individuals (“80–20” criterion) and 33 individuals (“90–10” criterion) of the 163 sampled (12.2% and 20.2%, resp.) were classified as hybrids. Focusing on the 20 hybrid individuals classified using the “80–20” criterion, hybrids were found as far west as the Roosevelt National Forest sampling locality in Colorado and as far east as the Carpenter Game Production Area along the Missouri River in South Dakota (Figure 1, Table 1). Using the more liberal hybrid classification criterion (“90–10”), the presence of hybrids extended east to the Newton Hills Game Production Area in South Dakota; the westernmost locality where hybrids were present was unchanged (Figure 1, Table 1).

3.2. Spatial Analyses. Generalized dissimilarity models (Figure 2) performed well as measured by the total variance explained and performed significantly better than models with random environmental variables. The full models explained 58.9% (“90–20” criterion) and 63.9% (“80–20” criterion) of the total observed variation in the similarity of the level of hybridization (Table 4). Models using only environmental variables explained about 6% less of the observed variation, and models using only geographic distance explained 38.8% (“90–10” criterion) and 39.9% (“80–20” criterion) of the variation. The GDM results based on the absolute difference in mean Q-scores were very similar (not shown) and are not discussed further.

The most important predictor variable in the full models was geographic distance, whereas these were Bio18 (precipitation of the warmest quarter) and elevation for models using only environmental variables (Figure 3). These results suggest that geographic distance and environmental heterogeneity along our sampling sites are partially correlated, but that geographic distance alone does not perform as well in explaining differences in the level of hybridization as environmental variables or the combination of distance and environment.

4. Discussion

Understanding the selective forces maintaining hybrid zones is an important component of understanding the evolutionary process generating and maintaining biodiversity. If natural selection in response to different environments during periods of allopatry contributed to divergence and speciation between closely related taxa, we might expect patterns of hybridization upon secondary contact to reflect the patterns of local environmental heterogeneity. In this study, we have shown that patterns of hybridization between \textit{Passerina amoena} and \textit{Passerina cyanea} are best explained by a model that includes both patterns of environmental variation across the Rocky Mountains/Great Plains ecotone and geographic distance between sampling localities (Figures 2 and 3, Table 4). Although the distance between sampling localities was clearly an important determinant of the differences in hybridization among populations, environmental variation alone was a more powerful predictor. This result held for both criteria we used to assess whether an individual was a hybrid (“80–20” and “90–10”).
Figure 3: Response curves for generalized dissimilarity models of the proportion of pure *P. amoena* individuals, plus one half of the hybrid individuals, in a population. Results are shown for “80–20” and “90–10” criteria, and for full models (with both geographic distance and environmental variables) and models using only environmental variables. Response curves in GDM are nonlinear, but constrained to be monotonic. The slope of each function is indicative of the rate of change in the proportion of hybrids along the environmental gradient concerned. The maximum height of a response curve indicates the relative importance of that variable in explaining the observed variation in the similarity between populations in the proportion of hybrids. The $y$-axes indicate fitted functions $f(x)$, where $x$ denotes the environmental variable concerned. Each of the functions is fitted as a linear combination of I-spline basis functions [41].
This pattern supports the findings of Swenson [29], who used a much different approach to explore the relationship between environmental heterogeneity and hybridization in Passerina buntings. Whereas our analyses focused on the patterns of hybridization within and among populations, the unit of hybridization in Swenson’s analysis was the geographic location of an individual determined to be a hybrid. The proportion of hybrid individuals in a population, which was central to our analysis, did not factor into Swenson’s work. Instead, Swenson solely focused on whether or not a hybrid had been recorded in a particular location and did not explore the similarities in the proportion of hybrids among populations as we did. Although the relevant environmental variables identified in our study (BioClim variables Bio10, Bio12, and Bio18, Table 4) were slightly different from the environmental variable with the greatest explanatory power in Swenson’s study (mean annual temperature, BioClim variable Bio1), the variables are correlated (Supplemental Table S2). For example, the correlation between Bio18 (precipitation during the warmest quarter), which had the greatest explanatory power in both of our “environment only” models (Table 4), and mean annual temperature (Bio1) was 0.506 (Supplementary Table S1). Despite the difference in methodologies between the studies, both found that environmental variation predicts patterns of hybridization, which strongly indicates that local environments influence the structure of the Passerina bunting hybrid zone.

At present, our data do not allow us to determine what is driving the relationship between hybridization and environmental variation, but we consider three general, not mutually exclusive possibilities. The first is that hybrid individuals may either prefer or avoid particular habitats, the second is that there may be differences in habitat-specific performance, and the third is that mate choice patterns could differ across the hybrid zone.

That the fitness of hybrid individuals may be related to environmental conditions is not new as it is central to the environmental gradient selection hypothesis of hybrid zone structure [43] and its close relative, the bounded-superiority hypothesis [14]. In both these hypotheses, the selection pressures faced by hybrid individuals depend on ecological context. It is important to note that hybrid individuals may or may not actively prefer particular habitats. It is possible that individuals might disperse some given distance and that any subsequent fitness consequences in a particular habitat may not be driven by choice.

Our results are consistent with the possibility that the environmental variation present across the Passerina hybrid zone somehow influences hybrid fitness. It has been argued that both the oriole and flicker hybrid zones (Icterus bulbicollis × Icterus galbula and Colaptes auratus auratus × Colaptes auratus cafer, resp.), which are also located in the eastern Rocky Mountains/western Great Plains ecotone, are maintained because hybrid individuals are restricted to particular habitats. In part due to differences in metabolic performance between the two species, Rising [44] suggested that I. galbula is better adapted to the more mesic environment characteristic of eastern deciduous forest and I. bullockii is better adapted to the more xeric environments that are more dominant in western North America. He further suggested that hybrids could only survive in the intervening ecotone [44]. Similarly, hybridization between the red-shafted and yellow-shafted subspecies of the Northern Flicker (C. auratus) appears to be strongly related to moisture patterns present across the same ecotone [14, 45]. Since mean temperature and precipitation of the warmest quarter (Bio10, Bio18) were important contributors in the GDM results (Table 4), it is possible that P. cyanea and P. amoena are differentially adapted to their respective habitats (more mesic for P. cyanea xeric for P. amoena) similar to what Rising hypothesized for Icterus [44]. Either through metabolic differences or some other mechanism, hybrid Passerina buntings may suffer greater fitness consequences on one or both sides of the hybrid zone. Irrespective of the exact mechanism, environmentally influenced differences in hybrid fitness suggest that natural-selection-driven adaptations to different habitats during the course of divergence between P. amoena and P. cyanea contributed to the speciation process, a hallmark of ecological speciation.

The third possible driver of the differences in hybridization relates to mate choice, which may depend on ecological context. If sensory bias plays a role in female mate choice [46] and the local environment influences a female ability to distinguish between con and heterospecific mates, the rate of hybridization may differ among populations. Although natal dispersal patterns are unknown for Passerina buntings [47, 48], if they do not differ among populations, then the relationship between environmental heterogeneity and hybridization observed could potentially result from differences in female mate choice. Previous work indicated that female buntings can readily, but not perfectly, distinguish between con and heterospecific males [19, 20], but it is not

<table>
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<th>Model</th>
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*Variables are shown in order of decreasing importance (Figure 3); dist: geographic distance; for an explanation of the bioclimatic variables, see Table 3.
known whether the local environment can influence female mate choice abilities.

Recent work has shown a significant narrowing and westward shift in the structure of the Passerina hybrid zone over the past 40–45 years [30]. Although climate data from WorldClim [38] are not of sufficient resolution to test this hypothesis, it is intriguing to speculate that changes in precipitation over the same time frame played an important role in the hybrid zone movement. Additionally, climate models suggest that annual precipitation will increase over much of North America, with the exception of the southwest, over the next 100 years [49]. Interestingly, the majority of the increase in annual precipitation is predicted to be driven by precipitation in the winter months (December, January, and February), whereas precipitation in the summer months (June, July, and August) is predicted to decrease [49]. If the structure of the Passerina hybrid zone is mediated through precipitation patterns during the warmest part of the year, as our data suggests, the zone may actually reverse course and shift eastward in the future as the ecotone becomes drier.

Regardless of the exact mechanism, it is clear that in order to fully understand hybridization and continued reproductive isolation between P. amoena and P. cyannea the ecological context within which the interactions between the species occur should not be ignored. Given that these species did not diverge sympatrically [22], they are likely adapted to different environmental conditions and these differences influence hybridization patterns. Consequently, the long-term stability of the hybrid zone, as well as the species themselves, depends at least in part on the stability of the patterns of environmental heterogeneity found in the eastern Rocky Mountains/western Great Plains ecotone.

Acknowledgments

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References


Pollinator-Driven Speciation in Sexually Deceptive Orchids

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Pollinator-mediated selection has been suggested to play a major role for the origin and maintenance of the species diversity in orchids. Sexually deceptive orchids are one of the prime examples for rapid, pollinator-mediated plant radiations, with many species showing little genetic differentiation, lack of postzygotic barriers, but strong prezygotic reproductive isolation. These orchids mimic mating signals of female insects and employ male insects as pollinators. This kind of sexual mimicry leads to highly specialised pollination and provides a good system for investigating the process of pollinator-driven speciation. Here, we summarise the knowledge of key processes of speciation in this group of orchids and conduct a meta-analysis on traits that contribute to species differentiation, and thus potentially to speciation. Our study suggests that pollinator shift through changes in floral scent is predominant among closely related species in sexually deceptive orchids. Such shifts can provide a mechanism for pollinator-driven speciation in plants, if the resulting floral isolation is strong. Furthermore, changes in floral scent in these orchids are likely controlled by few genes. Together these factors suggest speciation in sexually deceptive orchids may happen rapidly and even in sympatry, which may explain the remarkable species diversity observed in this plant group.

1. Introduction

The species diversity in the orchid family is extraordinary. There are more than 20 000 species in the orchid family [1], representing about 10 percent of angiosperm species. The remarkable floral forms found among orchids have always fascinated botanists and evolutionary biologists. Orchid species richness and the spectacular diversity of plant-pollinator interactions provide an exceptional opportunity for the study of pollinator-driven speciation. There is more and more evidence suggesting that pollinators play a major role in the generation of novel floral forms and the maintenance of species diversity in orchids [2, 3]. Sexually deceptive orchids are particularly suitable for investigating pollinator-driven speciation, because of their specialised pollination system. Here, using sexually deceptive orchids as examples, we systematically review what is known about the process of pollinator-driven speciation, examine the key factors that are essential for speciation processes, and discuss the possible modes of speciation in this orchid pollination system. In particular, we focus on the scenario of ecological speciation with gene flow.

Many theoretical analyses have suggested that three factors are of major importance in the process of ecological speciation: (i) disruptive selection, (ii) the strength of reproductive isolation, and (iii) the genetic basis of traits underlying reproductive isolation and traits under natural selection [4, 5]. For reasons detailed below and discussed elsewhere [2], nonadaptive speciation is unlikely to play a major role in the diversification of the well-investigated genera of sexually deceptive orchids, Ophrys and Chiloglottis. In this review, we therefore focus on these three factors in sexually deceptive orchids.

2. Sexually Deceptive Orchids

Pollination by deception is very common in orchids (about one third of orchids are deceptive) [1, 6], with sexual deception as an especially intriguing variety. Sexually deceptive orchids mimic the mating signals of female insects and employ male insects as pollinators, inducing them to engage in mating behaviour and pseudocopulation [7–9]. The chemical mimicry of sex pheromones of pollinator
females has been suggested to play a major role in this process [8–13]. Because insect mating signals are usually very specific [14], pollinator attraction by sexual deception is also very specific, each orchid only attracting one or very few insect species [15–17]. This specific pollinator attraction can therefore act as a reproductive barrier and prevent gene flow among species [7, 17–22].

Pollination by sexual deception is mostly found in orchids from Australia, Europe, South Africa, and South America [23–27]. However, sexual deception has recently been reported for the daisy Gorteria diffusa, the first confirmed case outside of Orchidaceae [28], indicating that this pollination mechanism may be more common than previously thought [29]. Among the different sexually deceptive orchids, the two best-studied genera are Chiloglottis (Diurideae) in Australia, and Ophrys (Orchideae) in Europe. In Chiloglottis, there are more than 30 species that are pollinated mostly by thynnine wasps of the genus Neozeleboria [15, 30–32]. In Ophrys, most of the more than 200 species are pollinated by species representing different genera of solitary bees, while a few species are pollinated by species of solitary wasps, flies, and beetles [17, 33, 34].

Although sexually deceptive orchids have been suggested to be a spectacular example of ecological speciation (with or without gene flow), few studies have systematically investigated key factors like selection and reproductive isolation. Instead, most studies have focused on mechanistic aspects of floral mimicry [8, 9, 35] or phylogenetic patterns [36, 37]. Here, we critically review the process of ecological speciation with gene flow for two orchid systems with sexual mimicry. Additionally, we conduct a meta-analysis focusing on traits that differ among closely related orchid species. For our meta-study, we extracted and combined data from previous studies on orchid phylogeny [22, 37], pollinator information [34], and phenotypic traits [33, 38]. We considered only species for which all three types of information is available. Pairwise species comparisons of phenotypic traits and geographic distribution [33, 38] within monophyletic groups, both within Ophrys and Chiloglottis [22, 37], were performed, because reproductive isolation and selection is required to prevent gene flow within these groups. Since the potential for speciation through hybridisation has been reviewed previously [35], we will not discuss this aspect here.

3. Pollinator-Mediated Selection in Sexually Deceptive Orchids

In angiosperms, floral and species diversity is thought to be driven by pollinator-mediated selection because of pollen limitation [39]. Pollen limitation has been shown in most flowering plants [3, 39–42]. In orchids, many pollination studies suggest that pollen limitation is widespread in the whole family [24, 43–45]. This is especially true for deceptive orchids [46], because pollinator learning may reduce pollinator visitation [47, 48]. Therefore, pollinators can impose strong selection on floral traits by mediating reproductive success and outcrossing [49]. Since morphology, sensory preferences, and behaviour differ among pollinator insects, selection imposed by them may lead to different adaptations [21, 49–51]. Floral traits involved in plant-pollinator interactions, such as phenology, scent, colour, and morphology, are likely under pollinator-mediated selection, since they are directly associated with pollination success (Figure 1) [2]. However, only one study has hitherto quantified phenotypic selection in a sexually deceptive orchid [52]. Another interesting yet noninvestigated aspect is density-dependent selection, a potential driver of pollinator shifts in this group of plants. Below, we summarise the traits likely under selection and highlight important targets for future investigations.

Pollinator-mediated selection on floral phenology can be seen as the need for synchronisation between flowering time and emergence time of pollinators [23, 48]. Because of their pollinators’ ability to learn to avoid deceptive flowers [33], most sexually deceptive orchids are probably predominantly pollinated by naive male insects [54]. This may impose strong selection on the flowering time of orchids. In other words, the flowering time of different orchid species can be regarded as an adaptation to the optimal time of pollinator activity. From our meta-study, about 8–10% of closely related species pairs of sexually deceptive orchids did not overlap in floral phenology (counted from Table S1 available online at doi:10.1155/2012/285081), suggesting disruptive selection on floral phenology among closely related orchid species. However, most species pairs do overlap in flowering time, the average degree of flowering time overlap being 59% and 47%, in Ophrys and Chiloglottis, respectively (Table 1).

In most sexually deceptive orchids, floral scent, which mimics the sex pheromones of female pollinators, has been suggested to be the main attractant of pollinators [8, 9, 20]; it is therefore likely to be under pollinator-mediated selection. Indeed, in both Ophrys and Chiloglottis, floral scent composition is driven towards their pollinators’ preferences in both quantity and quality [8, 9, 11–13, 15, 20, 55]. Furthermore, direct scent manipulation experiments showed that changing floral scent can dramatically reduce or increase pollinator visitation ([22] and Xu et al. unpublished data). Selection on floral scent is likely to be divergent among closely related species, which attract different pollinators with different floral scent compositions. In Chiloglottis, 95% of closely related species pairs produce a different type of floral scent and are pollinated by different pollinators (Table 1). The same is true for Ophrys, where 98% of closely related species pairs attract different pollinators (Table 1), most likely by the use of different floral odour bouquets [12, 17–20, 55–59].

Despite the importance of floral scent, floral colour and/or floral display may also play a role for effective pollinator visitation. In many species of Ophrys and Chiloglottis, the floral labellum (a modified petal) resembles the visual pattern of pollinator females, indicating that pollinators may use this visual signal to locate the flowers at short distance [38, 48]. Although it appears different to human eyes, the labellum of the Australian sexually deceptive orchid Cryptostylis is effectively identical in coloration to its pollinator females [60]. Many Ophrys labella may mimic the wings of a pollinator female with their shiny surface [16].
In long-horned bee-pollinated *Ophrys* species, the perianth colour and/or contrast with background can affect the short-range detectability of *Ophrys* flowers to their pollinators [61–63]. However, this does not appear to be the case in a *Colletes cunicularius*-pollinated *Ophrys* species [57], suggesting that the role of colour and/or display on pollinator attraction may vary among different pollinators and visual systems. In our metastudy, we did not include floral colour, because most descriptions of floral coloration are based on observations by human eyes and not on insect vision models.

After the pollinator has landed on a flower, floral morphology such as labellum shape, size, and texture may have a strong influence on pollinator behaviour. In orchids, precisely removing and delivering pollinia is highly dependent on the match of shape and/or size of the pollinators’ body and the floral labellum [48]. The significant correlation between pollinator body length and labellum length found in different *Ophrys* species clearly suggests that pollinator-mediated selection on floral labellum size is strong [48, 52, 64]. Selection imposed on floral labellum size may

### Table 1: Summary of comparisons of floral traits, distribution, and pollinators among closely related species pairs in *Ophrys* and *Chiloglottis*

Data on floral labellum, flowering time, and altitude range distribution are presented as mean (± standard deviation). Data on distribution, floral scent, and pollinators are presented as mean value.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Number of species-pairs compared</th>
<th>Overlap in labellum length (%) [33, 38]</th>
<th>Overlap in flowering time (%) [33, 38]</th>
<th>Overlap in habitat: Altitude (%) [33, 38]</th>
<th>Overlap in habitat: Area (%) [33, 38]</th>
<th>Overlap in floral scent (%) [22]</th>
<th>Overlap in pollinators (%) [34]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ophrys</em></td>
<td>213</td>
<td>42.6 (±31.7)</td>
<td>59.0 (±31.2)</td>
<td>83.0 (±19.2)</td>
<td>53</td>
<td>N/A</td>
<td>2</td>
</tr>
<tr>
<td><em>Chiloglottis</em></td>
<td>38</td>
<td>42.9 (±32.1)</td>
<td>47.4 (±34.6)</td>
<td>35.1 (±32.5)</td>
<td>74</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
be disruptive, since the body size of pollinators may vary considerably from each other. Indeed, the average overlap in floral labellum length among closely related species is only 43% in both *Ophrys* and *Chiloglottis* (Table 1), which suggests divergent selection on floral labellum size among closely related species.

**4. Reproductive Isolation in Sexually Deceptive Orchids**

Reproductive isolation among closely related sexually deceptive orchid species is mainly due to floral isolation, a form of pollinator-mediated prepollination reproductive isolation, while the postpollination reproductive barriers tend to be effectively absent or weak [21, 49] (but see [13]). For example, the closely related sympatric species *O. exaltata*, *O. garganica*, and *O. sphegodes* attract different solitary bees, *Colletes cumulans*, *Andrena pilipes*, and *Andrena nigroaenea*, respectively, and the lack of pollinator sharing leads to a highly effective barrier to gene flow in this case, although interspecies hand pollination experiments yielded normal seed set [18]. Interestingly, this apparent lack of postpollination reproductive barriers appears to be common in both *Ophrys* and *Chiloglottis* [22, 65, 66].

Since reproductive isolation in sexually deceptive orchids is mainly (if not only) mediated by floral isolation, the strength and stability of floral isolation are critical for pollinator-driven speciation. Although the hypothesis that floral isolation may effectively prevent gene flow between closely related species and thus drive speciation was already suggested half a century ago [16, 67], it was only recently that the strength of floral isolation was quantitatively measured *in situ* between species of the *O. sphegodes* group [18]. The results indicated that floral isolation among closely related species is very strong, with an isolation index higher than 0.98 [18]; a similar pattern was also found in another, unrelated *Ophrys* group (Gervasi and Schiestl, unpublished data), indicating that strong floral isolation is probably common in *Ophrys*. In *Chiloglottis*, pollinator behavioural tests on several sympatric species in different populations showed no cross-attraction, likewise suggesting strong floral isolation in this system [30, 66]. Furthermore, population genetic studies in both *Ophrys* and *Chiloglottis* suggest that floral isolation can effectively prevent gene flow between species in sympatry [18, 19, 66, 68–70].

Other studies based on genetic and phylogenetic analysis showed low genetic divergence among species in *Ophrys* and concluded that floral isolation might not be strong enough to prevent gene flow between species in sympatry [37, 71, 72]. However, low genetic divergence among species can be explained by different scenarios, such as recent radiations resulting in the retention of ancestral polymorphism [70]. Therefore, low genetic divergence among species cannot be taken as strong evidence of weak floral isolation per se. For a better understanding of the strength and variability of floral isolation, *in situ* measurements of floral isolation or systematic pollinator preference tests in more groups and populations over several years, or rigorous tests for hybridisation using molecular markers, are needed [73]. Furthermore, studies comparing neutral markers versus markers under disruptive selection will be important to shed light on the detailed mechanisms of speciation.

Floral isolation among closely related species of sexually deceptive orchids is either due to different pollinator attraction (ethological isolation) or different floral phenology (temporal isolation). Although mechanical isolation among species of the *Ophrys* sections *Pseudophrys* and *Ophrys* is evident [67, 74, 75], these groups are not closely related to each other and thus mechanical (morphological) isolation is unlikely to play an important role in their radiation (see discussion below). The key trait involved in ethological isolation is floral scent [21, 22, 56].

*Ophrys* flowers produce a complex mixture of more than 100 chemical compounds. Among these compounds, saturated and unsaturated hydrocarbons, that is, alkanes and alkenes, are responsible for pollinator attraction in many species [9, 11, 12, 18, 76]. Their specificity is due to quantitative variation in various alkenes with different double-bond positions and carbon chain lengths [9, 18, 19, 76, 77]. However, there are also some *Ophrys* species that employ only a few unusual chemical compounds for specific pollinator attraction. For example, in *O. speculum*, pollinator attraction can be achieved by the mixture of only eight compounds, in which two enantiomers of 9-hydroxydecanoic acid act as key substances [78]. Such signalling with few, specific chemical compounds is more common in *Chiloglottis*. Here, specific pollinator attraction is achieved by a single unusual compound (called “chiloglottone”) [8] or simple blends of chemically related chiloglottones [8, 22].

Besides floral scent, floral phenology and floral morphology may also play a role in floral isolation among closely related species. Some species pairs show no overlap in flowering time in sympatry, (e.g., *O. icericolor* and *O. mesaritica* on Crete [69, 79]). Among such species pairs, floral phenology may act as a strong reproductive barrier. However, as most closely related species pairs (~90%) of sexually deceptive orchids do overlap in their flowering times to a certain degree, floral phenology alone is usually not the primary reproductive barrier. Differences in floral labellum length between closely related species pairs may contribute to mechanical reproductive isolation as well. However, due to pollinator movements during mating attempts, variation in labellum morphology alone might not provide a strong barrier to gene flow. To better understand the evolutionary patterns of the respective reproductive barriers in sexually deceptive orchids, direct quantitative measurements of the contributions of different floral traits to reproductive isolation are needed.

**5. The Genetic Basis of Reproductive “Barrier Traits” that Are under Pollinator-Mediated Divergent Selection**

Understanding the genetic basis and complexity of divergent traits involved in reproductive isolation is important for understanding the process of pollinator-driven speciation,
6. The Process of Pollinator-Driven Speciation in Orchids

A key element of pollinator-driven speciation is that changes in pollinators are linked to reproductive isolation. While such pollinator shifts can in principle be brought about by a wide variety of floral trait changes, the importance of odour signals for pollinator attraction in sexually deceptive orchids makes floral scent the prime candidate for mediating pollinator shifts. Disruptive selection on odour phenotypes would lead to species divergence following an ecological speciation process [84, 85]. Since such odour traits may have a simple genetic basis [21, 22, 77, 81], this may also be a process of speciation with gene flow (or, “genic speciation”) [86–88]. Here, divergence occurs in spite of gene flow, while selection on a few loci in the genome is responsible for differences in pollinator attraction among species. The creation of population subdivision by pollinator-mediated selection may then lead to a buildup of larger islands of divergence in the genome brought about by an effective reduction of recombination, a process termed divergence hitchhiking [89–91]. At the same time, it should be noted that many orchid species have not reached the point where significant postmating barriers have accumulated [18, 65]. Overall, speciation by pollinator shift in sexually deceptive orchids may frequently be consistent with ecological speciation processes with gene flow.

The possibility for pollinator shifts is limited by the local availability of potential pollinators, which is subject to geographical variation, often referred to as the geographical pollinator mosaic [3]. The pollinator mosaic provides a route to allopatric and parapatric divergence scenarios [3], which are as applicable to sexually deceptive orchids as they are to other plants. However, unlike in many other pollination systems, sympatric speciation appears plausible in sexually deceptive systems [17, 19, 20, 22, 35, 69], the credibility of which is strengthened by the combination of strong floral isolation, divergent selection, and possibly a simple genetic basis for species differences in this system. Molecular data suggesting gene flow among sympatric species (e.g., [72]) have been taken as evidence against pollinator-driven speciation in sympatry [3]. However, (i) it is often difficult to distinguish between current gene flow and ancestral polymorphism [92, 93], and (ii) gene flow itself is consistent with genic and ecological speciation processes [86–88]. This also highlights the difficulty researchers face to conclusively demonstrate sympatric speciation, requiring the combined study of population genetics (or genomics), phylogeny, biogeography, and data on the strength and genetic basis of reproductive barriers between sibling species.

Ecological speciation processes with gene flow are not only consistent with sympatric modes of speciation, but also the local nature of species divergence implies that speciation processes may often result in progenitor-derivative species patterns rather than strict sister species relationships. Progenitor-derivative speciation (or “quantum speciation”) denotes the evolution of a new lineage (the derivative) from a source population (the progenitor) without affecting the progenitor [94–99]. Since pollinator shifts are not expected to change pollinator specificity in the source population, progenitor-derivative patterns are a likely outcome of pollinator-driven speciation in sexually deceptive orchids, with some genetic data supporting such scenarios [69, 70]. Despite the potential for sympatric and progenitor-derivative speciation, it remains to be seen whether these modes of speciation are common in sexually deceptive orchids,
our current knowledge being limited by the resolution of phylogenetic reconstructions among closely related sexually deceptive orchids (e.g., [37]).

7. Conclusions and Future Directions

Sexually deceptive orchids provide an exceptional study system for pollinator-driven speciation in plants. Our critical review and meta-analysis of traits in closely related sexually deceptive orchid species pairs suggest that pollinator shifts through changes in floral traits, especially floral scent, may be the main mechanism for speciation in this plant group. Such speciation through pollinator shift may happen rapidly and even in sympathy, if floral isolation is strong and floral trait changes are controlled by few genes. Therefore, to understand the detailed process of speciation in sexually deceptive orchids, it is necessary to identify the genetic basis of key floral traits and quantify their contributions to plant-pollinator interaction. Furthermore, to better understand the speciation patterns in this plant group, it would be important to evaluate their likelihood in a theoretical model under biologically plausible conditions based on empirical data [100, 101]. Such an integrative theoretical framework is still missing but is urgently needed to truly understand pollinator-driven speciation.

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References


Synergy between Allopatry and Ecology in Population Differentiation and Speciation

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The general diversity pattern of the Caribbean anole radiation has been described in detail; however, the actual mechanisms at the origin of their diversification remain controversial. In particular, the role of ecological speciation, and the relative importance of divergence in allopatry and in parapatry, is debated. We describe the genetic structure of anole populations across lineage contact zones and ecotones to investigate the effect of allopatric divergence, natural selection, and the combination of both factors on population differentiation. Allopatric divergence had no significant impact on differentiation across the lineage boundary, while a clear bimodality in genetic and morphological characters was observed across an ecotone within a single lineage. Critically, the strongest differentiation was observed when allopatry and ecology act together, leading to a sharp reduction in gene flow between two lineages inhabiting different habitats. We suggest that, for Caribbean anoles to reach full speciation, a synergistic combination of several historical and ecological factors may be requisite.

1. Introduction

Speciation, the mechanism at the origin of species diversification, is one of the most studied subjects in evolutionary biology. Despite this enormous interest, very little is known about the factors needed for speciation to occur. For instance, the relative importance of ecological versus purely historical factors, as well as the geographic context of speciation, is still debated, and mechanisms that are sometimes invoked to explain lack of speciation, such as observation of a species-area relationship [1], are themselves not fully understood.

The most widely recognised speciation model is the allopatric model, where different populations that are geographically isolated develop genetic incompatibilities, purely by genetic drift or founder effects [2–4], by adapting to different habitats (by-product speciation [5]), by sexual selection [6], or by fixation of incompatible mutations through adaptation to a similar habitat (mutation-order speciation [7]). Because there is no gene flow to counter the differentiation of populations, this model is the easiest to explain speciation, especially if the isolated populations are exposed to distinct selective environments (reviewed in [8]).

The possibility of speciation in the presence of recurrent gene flow (sympatric or parapatric speciation) is much more debated. In this model, the divergence between populations exchanging migrants is driven by ecological differentiation (ecological speciation [9]), and/or sexual differentiation (speciation by sexual selection [10]). Because even a limited amount of gene flow is expected to counter differentiation, early theoretical studies rejected this speciation model [11]. However, other theoretical and empirical work suggested that nonallopatric speciation is possible under particular circumstances (reviewed in [12]).

A group in which both the geographic context of speciation, and the role of ecological speciation, is debated is the Caribbean anoles. The anole radiation is a highly diverse species group useful for understanding the mechanisms at the origin of diversification. Anolis is one of the most
speciose vertebrate genera with more than 400 described species. Of these, ca. 150 are found in the Caribbean islands, and are thought to originate from just two independent colonizations from the mainland. This species diversity is accompanied by a very high morphological and ecological diversity. For these reasons, the anole radiation in the Caribbean has been the focus of intense studies in the last decades (reviewed in [13]).

One of the striking patterns in Anolis diversity is the contrast between the Greater and the Lesser Antilles. Whereas numerous species coexist within the large islands of the Greater Antilles, islands of the Lesser Antilles have at most two naturally occurring species with most of the islands having only one. Furthermore, if most of the diversification in the Greater Antilles occurred within island, most of the species pairs in the Lesser Antilles are not sister species, suggesting that these species pairs did not diverge within island but rather came together after dispersal from another island, with one possible exception in Saint Vincent [13].

The Caribbean anoles show a well-documented species-area relationship [14, 15]; however, the reason why so many speciation events happened in the Greater Antilles while almost none happened in the Lesser Antilles is still speculative. Losos and Schluter [14] proposed two explanations for this observation. First, larger islands could offer more opportunities of geographic fragmentation and hence lead to the formation of more species by allopatric speciation. Second, larger islands may have more habitat diversity, and hence populations submitted to divergent selective pressure in different habitats could lead to the formation of more species by ecological speciation. The observation that some of the largest islands in the Lesser Antilles, like Dominica, Guadeloupe, or Martinique, have a very high habitat diversity and hence should be able to support several species if ecological speciation was the driving force in anole diversification led Losos [13] to suggest that nonallopatric modes of speciation driven by ecological speciation are not supported in anoles.

Several studies suggest the opposite. For instance, because of its complex geologic history [16], the island of Martinique has offered plenty of opportunities for allopatric speciation to occur in its endemic anole, Anolis roquet. Present day Martinique was once formed of separate proto-islands where distinct mtDNA lineages of A. roquet evolved in allopatry for millions of years before coming back into secondary contact [17]. However, high gene flow is observed between previously allopatric lineages showing that this long geographic isolation did not lead to complete speciation [18]. Similarly, deep mtDNA lineages are observed in several species, both in the Lesser Antilles [19–23], and in the Greater Antilles [24–31]. In the cases where it has been studied, no restriction of gene flow has been detected between these previously allopatric lineages [18, 32, 33], with one possible exception in  North-Eastern Martinique [18]. All this suggests that even if allopatric speciation probably occurred in some situations, geographic isolation alone is not sufficient to reach speciation in anoles.

Instead, ecological gradients seem to be driving population differentiation in Martinique anoles. This island is very heterogeneous, both topographically and ecologically. The mountains in the North are exposed to the trade winds and receive a very high amount of precipitation all year round. At the opposite, the northern Caribbean coast is in the “rain shadow” of these mountains and is much drier and seasonal. Hence, the habitat changes dramatically from a cool montane rainforest to a hot xeric scrubland in just a few kilometres. Previous studies [18] have shown that the divergent selective forces along this habitat gradient lead to significant morphological and genetic differentiation between coastal and mountain populations of A. roquet. On the neighbouring island of Dominica, a similar situation appears to occur with its endemic anole, Anolis oculatus [32], and indeed, in the single case to date where contact zones have been studied in the Greater Antilles, a significant reduction in gene flow between divergent lineages is only observed in an area with a steep ecological gradient [33].

In this paper we reanalysed data from a previous study [18] and added a new transect of A. roquet populations where the ecotone and the lineage boundary overlap. We compared the population structure of this new transect to the two previously published ones, one between two lineages within a single habitat, and the other between two habitats within a single lineage. We studied the population structure and admixture rates along these different transects to investigate the effects of geographic isolation, ecological isolation, and the combination of these two factors on anole population differentiation and speciation. We observe that population differentiation is at its highest when both factors act simultaneously and suggest that a possible explanation of the species-area relationship observed in Caribbean anoles is that the probability of both allopatric and ecological factors acting in synergy increases with island size.

2. Material and Methods

2.1. Sampling. The distribution of anoles in Martinique is more or less continuous. Hereafter we use the term “population” to refer to a discrete sampling site, not to genetically or ecologically differentiated entities. We sampled three distinct transects in northern Martinique (Figure 1). First, the “lineage transect” (transect II in [18]) consisted of eight populations sampled in similar habitat (transitional forest) across the lineage boundary between the North-West (NW) and the Central (C) lineages. Second, the “habitat transect” (populations 1 to 6 from transect IV in [18]) consisted of six populations sampled within the NW lineage and across an ecotone between coastal scrubland and montane rainforest. Finally, the “combined transect” (new data) consisted of eight populations sampled across the lineage boundary between the NW and the C lineages and across the ecotone between coastal scrubland and montane rainforest. For each population, tail tips from 48 individuals were sampled, and quantitative traits measurements (see below) were collected on ten adult males.

2.2. Genetic Analyses. For each individual, genotypes were scored at nine microsatellite loci (AAE-P2F9, ABO-P4A9,
Mitochondrial DNA

(i) Admixture Analysis. We estimated the genetic structure within each transect using the Bayesian clustering method implemented in STRUCTURE v2.3.2 [37]. First, we estimated the most likely number of clusters (k) by running the analysis with k ranging from 1 to the real number of populations. For each k value, the analysis was run 10 times using the admixture model, with a burn-in of 100,000 steps for a total run length of 500,000 steps. The optimal number of clusters (K) was inferred following the method outlined for a total run length of 500,000 steps. The optimal number of clusters (k) was estimated using the admixture model, with a burn-in of 100,000 steps for a total run length of 500,000 steps. The optimal number of clusters (K) was inferred following the method outlined

(ii) We then estimated the influence of different potentially important factors on the genetic structure using the method implemented in GESTE v2.0 [41]. GESTE employs a hierarchical Bayesian framework to estimate population specific Fst (representing the differentiation of a given population relative to all other populations) and uses a generalized linear model in order to test the contribution of biotic or nonbiotic factors to genetic structuring. For our analyses we used the default settings (sample size of 10000, thinning interval 20), and in accordance with guidelines we allowed ten pilot runs to estimate means and variances for the required input parameters [41]. The analysis was repeated five times for each transect to ensure that the results were consistent. Three different factors were considered. First 19 bioclimatic variables were obtained for each population from the WorldClim database. These variables were annual mean temperature, mean diurnal range, isothermality, temperature seasonality, maximum temperature coolest month, minimum temperature hottest month, temperature annual range, mean temperature wettest quarter, mean temperature driest quarter, mean temperature warmest quarter, mean temperature coolest quarter, annual precipitation, precipitation wettest month, precipitation driest month, precipitation seasonality, precipitation wettest quarter, precipitation driest quarter, precipitation warmest quarter, and precipitation coldest quarter. A principal component analysis (PCA) of the log transformed variables was performed using the ade4 package [42] in the R environment [43]. The first component explained more than 75% of the variation and was used as a composite bioclimatic variable to describe the environment among each site. This first axis described the variation from hot and seasonally dry coastal sites to cool and wet montane sites. Second, the geographic connectivity of the populations was estimated as described in [44] to include a measure of geographic isolation for each individual population (mean geographical distance between a given population and all other populations). Third, each site was assigned to a lineage (lineage transect), a habitat (habitat transect), or both (combined transect) as follows. For the lineage transect, sites 1–3 were assigned to the NW/coastal group, and sites 4–8 were assigned to the C/montane group. For the habitat transect, sites 1–3 were assigned to coastal habitat, and sites 4–6 were assigned to montane habitat. For the combined transect, sites 1–3 were assigned to NW/coastal group, while sites 4–8 were assigned to C/montane group.

(iii) Transects Comparison. To determine if the genetic structure was different among transects, we computed and plotted the pairwise Fst’ values between populations on each side of the contact zone within each transect. This allowed us to compare the level of across habitat/lineage differentiation among transects. Because the observations are nonindependent and hence violate the assumption of both parametric and nonparametric tests, these plots provide qualitative information that has not been tested statistically.

2.3. Combined Analysis of Genetic and Quantitative Data. We conducted a modified version of the Discriminant Analysis of Principal Components (DAPC, [45]) to describe the global structure of populations within each transect using both genetics and morphology.
First different morphological characters were recorded as previously described [22, 46]. These included body dimensions (jaw length, head length, head depth, head width, upper leg length, lower leg length, dewlap length), scation (number of postmentals scales, scales between supraorbitals, ventral scales, and dorsal scales), colour pattern (number of dorsal chevrons, chevron intensity, occipital “A” mark, back dorsal reticulation, white spots), and trunk background colour (percentage red, green and blue hue on the posterior trunk). These were combined to six independent hues (UV 330–380 nm, UV/violet 380–430 nm, blue 430–490 nm, green 520–590 nm, yellow/orange 590–640 nm, and red 640–710 nm) extracted from the spectrum of the anterior and posterior dewlap by a multiple-group eigenvector procedure [23]. This combined data set was subjected to a principal component analysis (PCA) using the ade4 package in R.

Then, the microsatellite data were also subjected to a PCA using the adegenet package [47] in R. Finally, the components from the genetic (14 components) and quantitative (4 components) datasets were combined and subjected to a linear discriminant analysis using the MASS package [48] in R, using the population of origin as the grouping factor.

3. Results

3.1. Lineage Transect. Two genetic clusters were identified in this transect, with individuals from populations 1 to 3 being assigned in majority to the first cluster and individuals from populations 4 to 8 to the second cluster (Figure 2(a)). This separation corresponds to what was observed with mitochondrial DNA, populations 1 to 3 being in majority from the NW lineage while populations 4 to 8 are in majority from the C lineage [18, 46]. However, the proportion of admixed individuals is very high and relatively constant all along this transect (between 35 and 56%), and there is no trend in the pairwise Fst’ that vary between 0.072 and 0.101 (Figure 2(a)). Morphological and genetic data analysed separately (Figure S1) show the same trend that is magnified in the combined analyses. We observe a slight differentiation between populations 1 to 3 and populations 4 to 8, but there is an overlap between these groups (Figure 3(a)). The population specific Fst estimated with GESTE (Table 1) that represents the level of differentiation of one population relative to all other [41] are very low (ranging from 0.008 to 0.013, Table 1) and do not correlate with any of the factors investigated (geographic connectivity, environment, lineage), underlining again the lack of genetic structure along this transect (Table 2).

3.2. Habitat Transect. Two genetic clusters were also identified in this transect (cluster 1: populations 1–3; cluster 2: populations 4–8, Figure 2(b)). This genetic division corresponds to the habitat division, the ecotone being situated between populations 3 and 4 [18]. The admixture rates are globally lower than on the lineage transect (range: 15–55%). It is relatively low at the two opposite sides of the transect (29% in population 1 and 15% in population 6) and gets higher in the centre of the transect, around the ecotone (55% in population 3 and 45% in population 4). Here again, there is no obvious trend in the pairwise Fst’ that vary between 0.059 and 0.112 (Figure 2(b)). The combined dataset shows a much higher level of variation than on the lineage transect (almost twice as high), and a marked differentiation between populations 1-2 and 4–6, with population 3 being intermediate (Figure 3(b)). The population specific Fst are higher than in any site of the lineage transect (ranging from 0.0148 to 0.0522, Table 1) confirming the higher genetic structuring along this transect. The best model to explain this genetic structure only incorporates a constant, but the second and third best models have a nonnegligible probability and incorporate, respectively, the habitat type and the bioclimatic variable (Table 1); these two factors have a combined probability of 0.175 and 0.134, respectively (Table 2).

3.3. Combined Transect. Here again two genetic clusters were identified (cluster 1: populations 1–3; cluster 2: populations 4–8, Figure 2(c)), but with a much higher differentiation. This genetic division corresponds both to the habitat and lineage boundaries, the ecotone being situated between populations 3 and 4, as well as the lineage boundary (Figure 4). On this transect, the admixture rate is much lower (range 4–27%) than on the other transects. It is somewhat higher in population 4, situated at the lineage boundary and at the ecotone, but even in this population, it is lower than in any of the lineage transect’s populations and than in most of the habitat transect’s populations (Figure 2(c)). There is also a marked increase of the pairwise Fst’ at the contact zone, with a value of 0.169 between populations 3 and 4, while the other values are much lower (range: 0.052–0.0760, Figure 2(b)), suggesting the existence of a barrier to gene flow at the lineage/habitat boundary. The combined dataset shows a similar level of variation than in the habitat transect, with a strong differentiation between coastal (1–2) and montane (5–8) populations, with the populations 3 and 4 being intermediate. Along this transect, the population specific Fst are similar or higher to what is observed in the habitat transect (Table 1). The genetic structure is significantly associated with the habitat type/lineage factor, and the second best model includes the bioclimatic variable with a nonnegligible probability (Table 2).

4. Discussion

In this paper, we describe the differentiation between two lineages of *Anolis roquet* living in contrasting habitats and coming into secondary contact at the ecotone between these habitats. As a comparison, we reanalysed two previously published transects, a “lineage transect” where two lineages meet within a same habitat, and a “habitat transect” where different populations of the same lineage live in contrasting environments and are in contact at the ecotone between these habitats. This design allowed to investigate the effects of allopatry, habitat, and of the combination of these two factors on anole population differentiation and speciation.

A marked difference could be observed in the population structure along these three transects (Figure 5), suggesting
Figure 2: Population structure and admixture rates along the three transects. Solid lines represent admixture rates, and broken lines represent pairwise Fst values.

Figure 3: DAPC (discriminant analysis of principal components) based on the combination of genetic and quantitative trait characters (see supplementary Figure S1 available online at doi: 10.1155/2012/273413 for the separate analyses).
Table 1: Bayesian estimates of Fst values for each population. The mean value and the lower and upper limits of the 95% highest probability density interval are indicated. These Fst values measure the differentiation of a given population relative to all other populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Lineage transect</th>
<th>Habitat transect</th>
<th>Combined transect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0078 [0.0038; 0.0123]</td>
<td>0.0522 [0.0357; 0.0695]</td>
<td>0.0721 [0.0554; 0.0934]</td>
</tr>
<tr>
<td>2</td>
<td>0.0104 [0.0055; 0.0155]</td>
<td>0.0464 [0.0301; 0.0627]</td>
<td>0.0513 [0.0357; 0.0674]</td>
</tr>
<tr>
<td>3</td>
<td>0.0054 [0.0019; 0.0093]</td>
<td>0.0330 [0.0209; 0.0468]</td>
<td>0.0507 [0.0350; 0.0671]</td>
</tr>
<tr>
<td>4</td>
<td>0.0097 [0.0049; 0.0151]</td>
<td>0.0148 [0.0071; 0.0228]</td>
<td>0.0148 [0.0075; 0.0223]</td>
</tr>
<tr>
<td>5</td>
<td>0.0061 [0.0022; 0.0101]</td>
<td>0.0219 [0.0122; 0.0323]</td>
<td>0.0131 [0.0064; 0.0206]</td>
</tr>
<tr>
<td>6</td>
<td>0.0132 [0.0075; 0.0193]</td>
<td>0.0233 [0.0142; 0.0339]</td>
<td>0.0134 [0.0071; 0.0205]</td>
</tr>
<tr>
<td>7</td>
<td>0.0090 [0.0044; 0.0139]</td>
<td>0.0122 [0.0060; 0.0190]</td>
<td>0.0136 [0.0066; 0.0210]</td>
</tr>
<tr>
<td>8</td>
<td>0.0101 [0.0052; 0.0153]</td>
<td>0.0170 [0.0103; 0.0237]</td>
<td>0.0182 [0.0105; 0.0259]</td>
</tr>
</tbody>
</table>

Table 2: Environmental factors determining the genetic structure of populations. (a) Sum of posterior probabilities of models including a given factor. (b) Posterior probability of the 8 models considered. The best model is indicated in bold. (c) Estimates of the regression parameters for the best model (mean value and lower and upper limits of the 95% highest probability density interval are indicated).

<table>
<thead>
<tr>
<th>Lineage transect</th>
<th>Habitat transect</th>
<th>Combined transect</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connectivity (G1)</td>
<td>0.069</td>
<td>0.107</td>
</tr>
<tr>
<td>Environment (G2)</td>
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<td>0.184</td>
</tr>
<tr>
<td>Lineage/habitat (G3)</td>
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<td>0.228</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td><strong>0.800</strong></td>
<td><strong>0.554</strong></td>
</tr>
<tr>
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<td>0.057</td>
<td>0.067</td>
</tr>
<tr>
<td>Constant, G2</td>
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<td>0.134</td>
</tr>
<tr>
<td>Constant, G3</td>
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<td>0.175</td>
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<tr>
<td>Constant, G1, G3</td>
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<td>0.020</td>
</tr>
<tr>
<td>Constant, G2, G3</td>
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<td>0.030</td>
</tr>
<tr>
<td>Constant, G1, G2, G3</td>
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<td>0.003</td>
</tr>
<tr>
<td>(c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>0.75 [-1.22, -0.29]</td>
<td></td>
</tr>
<tr>
<td>$\sigma_2$</td>
<td>0.39 [0.10; 0.84]</td>
<td>0.70 [0.13; 1.63]</td>
</tr>
</tbody>
</table>

that they are at different stages of the speciation continuum [49, 50]. According to Hendry et al [49], four stages can be distinguished along this continuum: “(1) continuous variation within panmictic populations, (2) partially discontinuous variation with minor reproductive isolation, (3) strongly discontinuous variation with strong but reversible reproductive isolation and (4) complete and irreversible reproductive isolation.” The weak structure observed in the lineage transect, and the high admixture level correspond the State 1 of the continuum. This pattern is similar to what was observed in a previous study [18] for all but one (transect I in [18]) transects sampled across lineages (transects II, III, IV, V, VI, VII, VIII in [18]). In the habitat transect, the variation is clearly bimodal, but the high level of admixture in the contact zone between the two habitats suggests that the reproductive isolation is still limited between the ecotypes, corresponding to a stage between State 2 and State 3 of the continuum. Here again, this pattern is similar to the other transect sampled across habitats (transect III in [18]). Finally, the combined transects present strongly discontinuous variation, but there is still a significant level of admixture at the contact zone suggesting that the reproductive isolation is not complete despite a strong reduction in gene flow. This would correspond to State 3 of the speciation continuum. The situation of this last transect, where the ecotone and the lineage boundary overlap, is unique on this island, and hence this result could not be replicated.

In terms of association between observed genetic structure and biotic and abiotic factors, no significant factors were detected on the lineage transect, all the models other than the one incorporating only a constant having a very low probability. To the contrary, for the combined transect both the habitat/lineage category and the bioclimatic composite variable are the best at explaining the genetic structure, suggesting the important driving force of the environment into population differentiation. The situation in the habitat transect is not so clear. The best model only incorporates a constant, but the combined probability of the habitat type and the environment factor are not negligible as they were on...
the lineage transect. The lack of significance of these factors could be due to the lack of power of this method when the number of populations analysed is low. Indeed, simulations showed that this method failed to identify the true model with five or seven populations [41]. However, the observed trend suggests that environment factors may play a role in the genetic structuring of the populations along this transect too.

As expected, we find the strongest signal of divergence in morphological data that are known to react quickly to selection [51–54] (Figure S1). The genetic data is similar in pattern but smaller in magnitude. Recent simulation studies suggest that neutral markers, that is, markers that are not linked with the traits under selection, are not very sensitive to detect ecological speciation [55, 56]. The authors conclude that this would lead to false negatives (failure to detect ecological speciation) rather than false positives. Taking this into account, and the fact that we found a clear signal of gene flow reduction both in the habitat and the combined transect in accordance with an extreme environment gradient, the divergence we demonstrate in this study with these neutral markers is undoubtedly considerably less than the divergence of the traits and loci under selection.

Since the habitat along the lineage transect is very homogeneous we do not expect ecological speciation to currently play a role in this area. However, this does not mean it has always been the case. When the populations from the two lineages were isolated on different proto-islands, it is possible that they were submitted to different environmental pressures. In such a situation, several studies have demonstrated that isolated populations can rapidly evolve partial reproductive isolation as a byproduct of local adaptation [57–60]. Such a mechanism could also explain the differences observed between the three transects. When populations from the C and NW lineages came back into secondary contact, they did so in two very different contexts: either along a very sharp environmental gradient (combined transect), where any preexisting reproductive isolation could be maintained or strengthened by current disruptive selection, or within an homogeneous habitat (lineage transect) where any preexisting reproductive isolation may have been lost. Breakdowns of reproductive barriers associated with ecological changes have been recently described in various species [61, 62].

The relative role of geography and ecology in speciation remains a subject of debate. The main discussion relates to whether or not ecological factors can drive speciation in the presence of gene flow. Several convincing empirical studies suggest that it is indeed possible to reach full speciation in sympatry by ecological speciation (e.g., [63–67]), while others emphasize the combined role of historical and ecological factors in shaping species diversity [68–70]. For instance, in Trinidadian guppies, ecological speciation played a role in premating isolation either in allopatry (byproduct speciation) or in parapatry (to avoid maladaptive matings) [69]. For Martinique anoles, it is clear that for the populations that reached secondary contact very little evidence of the role of geographic isolation exists, while ecological factors seem to play a more important role. However, without conducting mate-choice experiments, it is not possible to determine conclusively the role of ecological speciation in allopatry.

Despite the large number of studies on the Caribbean anole radiation, very little is known about the factors at the origin of their diversity. Several papers have described the diversity patterns, demonstrating a correlation between island size and species diversity in large islands, while no speciation events were recorded on islands below a threshold size [13]. Recent work demonstrated that within island diversity could be the result of ecological opportunities and that net speciation rate decreased with time as opportunities decreased to reach an equilibrium at the island carrying capacity [15]. However, these studies do not explain the mechanisms at the origin of these diversity patterns and only propose several hypotheses to explain these observations.

In line with previous studies, we demonstrate that indeed environmental factors have a strong effect on the genetic structure of Martinique anole populations, with a reduction
of gene flow at the ecotone between coastal and montane habitats and that it does not seem sufficient to lead to full reproductive isolation. Furthermore, we refine our understanding of divergence in these anoles by demonstrating that when allopatric lineages come into secondary contact on an ecotone, the differentiation is much stronger, with a significant reduction in gene flow. The absence of replication of the combined transect does not allow the generalisation of these findings, but we hypothesise that to reach full speciation anoles need first to evolve in allopatry and then come into secondary contact in an area where divergent natural selection will allow them to stay separate and further reinforce their divergence. Because this combination of factors is more likely to be found on large islands, it could be the mechanism at the origin of the species-area relationship observed in Caribbean anoles.

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References


[63] E. Rolan-Alvarez, M. Carballo, J. Galindo et al., “Nonallopatric and parallel origin of local reproductive barriers between two...


Review Article

Of “Host Forms” and Host Races:
Terminological Issues in Ecological Speciation

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Successful communication and accurate inferences in science depend on the common understanding and consistent usage of the terms we apply to concepts of interest. Likewise, new terminology is required when important concepts have gone unnamed. Here, I focus on terminological issues about biological variation and ecological speciation, especially in herbivorous insects but also more generally. I call for the more restricted use of concepts that have sometimes been misapplied, and thus caution against synonymizing ecological speciation with sympatric speciation and the unwarranted invocation of “host races” to describe herbivorous insect differentiation. I also call for the qualified application of terms for different kinds of biological variation and for host range when confronting uncertainty. Among other “missing terms” introduced here is “host form,” a generic term describing any case of host-associated differences for which current evidence does not allow diagnosis of the specific kind of variation. Embracing the use of host form should free host race from its current overapplication. Finally, I present a case study in which Neochlamisus leaf beetle populations previously described as host forms are hereby declared to be host races, based on accumulated evidence supporting each of the associated criteria.

1. Introduction

A rose by any other name may indeed smell as sweet. However, if a rose vendor started advertising his product as “ragweed,” he would find himself selling fewer flowers. And if a biologist unfamiliar with ragweed purchased his wares for analysis, they would come to different conclusions than had they collected actual ragweed in the field. Words, after all, are imbued with the meanings we assign to them. In science, effective communication entails that terms have well-defined and consistently used meanings and that new terms be created as needed to describe unlabelled concepts. In a field as concept-driven as evolutionary biology [1, 2], maintaining a one-to-one relationship between terms and meanings is especially critical. Indeed, our rose merchant’s application of a different “common name” to his roses need not have confused our biologist if their scientific name was also on display. This is because Linnaeus’s stratagem of applying a unique Latin binomial to every species [3] provided a common and specific language that eliminated confusion about what organism two people might be discussing, even if they differed in nationality or native tongue.

The Linnaean system of binomials continues to be usefully applied today. However, the typological view of species on which it was based primarily retains its utility for the taxonomist. Following Darwin’s Origin [4], the typologist’s notion of species as largely invariant and discretely different entities gave way to a population-oriented view that embraced continuous intraspecific variation and invoked reproductive barriers to genetic exchange to explain how species remained differentiated from each other [5]. This “population thinking” and the associated biological species concept promoted the recognition of various kinds of biological variation within and around the species level and the consideration of how they relate to species diagnosis and the process of speciation [5]. For example, the entomologist Benjamin Walsh recognized quite early that causal relationships likely existed among the host plant associations of herbivorous insects, their patterns of phenotypic variation, their opportunities for interbreeding, and their species status.
He thus spoke of “phytophagic varieties” and “phytophagic species” [6]. In so doing, Walsh began a long tradition of using herbivorous insects as exemplars of different kinds of biological variation [7–10] and for the study of speciation [2, 22, and 34]. Most recently, insect herbivores have provided many of the best-documented examples of what has become known as ecological speciation [11, 12].

Here I address outstanding terminological issues that affect our understanding of biological variation and ecological speciation and that are currently in need of attention. In illustrating such issues I focus largely on herbivorous insects for the reasons given above. That said, I additionally introduce new terms that are applicable to all taxa. A major goal is to demonstrate that, far from being merely semantic in nature, terminological shortcomings have important consequences for how we view central issues in speciation. I thus further call for the careful and specified use and creation of terms. I do not, however, chronicle and count up cases of terminological misuse in the literature to support my assertions of their existence. I do not wish to unfairly single out particular authors for misnomers common to others. And I do not believe that a more systematic evaluation of misuse frequencies is necessary to argue the value of a more stringent use of terms. Rather I hope that by considering the points made here the reader will develop a more critical eye in reading and writing the associated literature, to the betterment of speciation studies.

2. Ecological Speciation ≠ Sympatric Speciation

To begin let us consider a recurring misuse of “ecological speciation” itself. First I give some background.

For much of the 20th century a principal focus of speciation studies was determining the geographic context in which speciation occurred and the relative efficacy of different contexts for facilitating this process [13]. An issue of special concern was how readily populations could speciate in the absence of geographic isolation (allopatry), with the view becoming dominant that nonallopatric, “speciation in the absence of geographic isolation (allopatry), with the special concern was how readily populations could speciate and recombine. Thus, while enthusiasm for sympatric speciation and sympatric speciation received special scrutiny as it required that reproductively isolated populations evolve from within an initially panmictic population in the face of opportunities for homogenization by the countervailing forces of gene flow and recombination. Thus, while enthusiasm for sympatric speciation has continued to wax and wane, it is generally accepted to be a theoretically more onerous mode than allopatric speciation and thus to require quite particular conditions for its occurrence [14, 15].

Toward the end of the 20th century, debates about geographic modes had to make room for an emerging interest in the causes of reproductive isolation per se, and especially in the potential contributions of ecological adaptation and sexual selection. The term “ecological speciation” was coined to describe the evolution of reproductive isolation as an incidental consequence of divergent adaptation to alternative environments, due to the pleiotropic effects of selected genes, or the direct effects of genes tightly linked to them, on traits involved in reproductive isolation [16, 17]. As experimental and comparative evidence for the association of ecological traits with reproductive isolation accumulated, ecological speciation gained support as an evolutionarily important process and has induced little controversy [18, 19] but see [20].

Critically, ecological speciation is not dependent on geographic context. So long as reproductive isolation is evolving as a consequence of divergent adaptation, ecologically speciating populations could be completely allopatric on separate islands or completely sympatric, living on different plant species intermingling in the same field. Similarly, sympatric speciation may depend on ecological differentiation, or alternatively on sexual selection or polyploidy [21]. Thus, ecological speciation and sympatric speciation incompletely overlap in the cases of speciation they circumscribe [22]. Nonetheless, the intermingling plant scenario exemplifies the reliance of many models of sympatric speciation on the ecological divergence of newly formed subpopulations. And if indeed sympatric speciation is rarer than allopatric speciation and most commonly involves ecological divergence, then most cases of sympatric speciation will also represent ecological speciation, while only a minority of ecological speciation scenarios will involve sympatric speciation.

Despite this asymmetry, ecological speciation is sometimes equated (confounded) with sympatric speciation in the literature. Sometimes an association between ecological divergence and reproductive isolation is taken as de facto evidence for sympatric speciation in the absence of support for sympatric origins. This presumably occurs because the commonly ecological basis of sympatric speciation has forged a false equivalency between ecological causes of reproductive isolation and sympatric speciation in the minds of some workers. In the extreme, the term ecological speciation itself is sometimes coopted as a synonym for sympatric speciation and even erroneously contrasted with allopatric speciation.

This terminological misuse is important as it contributes to misunderstanding about the relative frequency of speciation mechanisms. Ecological speciation per se is theoretically simple and straightforward, and abundant empirical evidence now supports it [18], while conditions for sympatric speciation are theoretically restrictive and evidence compellingly supporting it—regardless of how common it may actually be—is difficult to obtain [14]. Thus, conceptually equating these terms makes sympatric speciation appear to be more broadly supported than it actually is.

3. The Kinds of Biological Variation

Speciation is a population-level process with many stages, and species are heterogeneous entities. Thus, their study requires a lexicon of terms describing variation at the individual, population, and species levels, one that is sufficient to describe any case of biological variation. In herbivorous insects, these kinds of variation are often host specific. They may also be cryptic, as host adaptation may be behaviorally or physiologically rather than morphologically based. Not for nothing were herbivorous insects focal subjects for early discussions of sibling species [7, 8] and later evaluations of intraspecific ecological heterogeneity [23]. These many
sources of variation led to terminological confusion and a need for clear distinctions [10]. The following list of terms is a bit entomocentric and is not comprehensive (missing, e.g., various infraspecific botanical terms), but should nonetheless allow for the assignment of differences between two organisms of any taxon to one or more kinds of biological variation.

**Species.** A contentious term with many suggested definitions (i.e., concepts; see [21]). These generally describe a set of populations that is reproductively, historically, genetically, and/or ecologically distinct from other such populations. The biological species concept, whereby species are groups of actually or potentially interbreeding populations that are reproductively isolated from other such groups, remains the most frequently adopted definition by evolutionary biologists.

*Sibling species (sensu [13]) = cryptic species.* Biological species that are approximately anatomically identical to each other. Herbivore sibling species are commonly associated with different host plants.

**Hybrids (sensu [24]).** The products of interbreeding between differentiated populations or separate species.

**Geographic races = subspecies (cf. [24]).** Geographically distinct populations exhibiting genetically based phenotypic differences. Subspecies are essentially geographic races with formal taxonomic status.

**Ecotypes (sensu [25]) (related to the above).** Spatially distinct populations exhibiting divergent adaptation to alternative environments. Herbivores adapted to different host plants in different areas are an example.

**Host races (sensu [26]).** Sympatric populations that are incompletely reproductively isolated but remain ecologically differentiated in the face of gene flow due to divergent selection on populations using alternative hosts. Generally applied to herbivores, but sometimes to other parasites. The diagnosis of host races is discussed below.

**Sympatric races.** A “missing term” (coined here) completely analogous to host races but applied to populations of any taxon—namely, nonparasitic ones—using analogous criteria.

**Populations (sensu [13]).** Ideally, panmictic groups of individuals. More commonly used to refer to spatially coherent groups of potentially interbreeding individuals that exhibit reduced migration and thus potential genetic differentiation.

**Envirotypes.** A “missing term” (coined here) referring to populations or individuals that differ in phenotype due to differences in environmental rather than genetic factors, that is, reflecting phenotypic plasticity. Examples are provided by induced differences between conspecific herbivores as a function of the different host plants on which they developed.

**Morphs (sensu [13]).** Discrete phenotypic variants that segregate within a population.

**Polymorphisms (sensu [27]).** Multiple genetic variants, often segregating within a population (includes morphs).

**Biotypes (sensu [10]).** The set of herbivorous insect individuals that share the same genotype at given loci of interest and thus phenotypically differ from other biotypes. Most often used in reference to different insect herbivore genotypes, each adapted to use a different host plant genotype.

**Sexual dimorphism.** Phenotypic differentiation between the sexes within a population.

**Developmental variation.** Differences in phenotype reflecting different stages of individual development or of a complex life cycle.

**Host forms.** A “missing term” (coined here) for groups of herbivore individuals or populations exhibiting host-associated biological variation, but where the kind of variation has not yet been diagnosed.

**Ecological forms.** A “missing term” (coined here) completely analogous to host forms but appropriately applied to members of any taxon—namely, nonparasitic ones—that exhibit habitat-or resource-associated differentiation.

Excepting host races, discussed below, means of diagnosing these kinds of variation are not described here.

### 4. Nominal Host Ranges

For insect herbivores another sort of variation is critical for understanding ecological speciation: host range, that is, the number, identity, and relatedness of host plant taxa [28]. Variation in host range is described by its own suite of terms. A “narrow” host range includes few and/or closely related hosts, while a “broad” host range includes many and/or distantly related host taxa. The narrower its host range, the more “specialized” a herbivore is said to be, with insects using a single host taxon being described as “monophagous.” The broader the host range, the more “generalized” or “polyphagous” the herbivore is said to be. Herbivores using several host taxa are referred to as “oligophagous.”

An accurate understanding of a herbivore’s host range is crucial to studying host-associated ecological speciation. Notably, most such examples involve highly specialized populations that are divergently adapted to alternative host plant species [11, 12]. Thus, one might predict a greater tendency towards specialization in a herbivore group to be associated with a greater tendency towards ecological speciation, via host shift and subsequent adaptation to the new host. One might also predict a herbivore taxon whose...
species tend to specialize on distantly related host plants to be more prone to ecological speciation than one whose species all use closely related hosts. This is because host shifts between more biologically divergent hosts will likely incur more strongly divergent selection, resulting in concomitantly large increases in reproductive isolation [29]. *Neochlamisus* leaf beetles, my focal study system, provide an example of both of these tendencies. Species in this genus usually specialize on a single host genus or species, with these hosts broadly scattered across eudicot phylogeny [29]. Following the above predictions, the only *Neochlamisus* species that regularly uses multiple (six) different and distantly related host genera/species, *N. bebbiana*, exhibits pronounced host-associated ecological divergence and reproductive isolation between populations affiliated with different hosts [29]. In this example, what was described as an oligophagous species [30] was experimentally proven not to be composed of populations with similar oligophagous tendencies, but rather of a complex of divergently host-specialized populations, each accepting its native host (i.e., the one used in nature) more readily than the five plants used by other populations [31].

The case of *N. bebbiana* not only illustrates why accurately assessing host range is vital to understanding ecological speciation but also why many herbivore host ranges are likely to be inaccurately or incompletely documented. Identifying the particular suite of hosts used by a given species or population requires intensive fieldwork. This includes the inspection of presumed nonhosts cooccurring with documented hosts, to determine if host range is broader than presumed. It further requires evidence that a “documented” host is indeed actively used as resource rather than simply being a place of temporary rest by an insect “tourist,” in which case the actual host range is narrower than presumed. Moreover, as with *N. bebbiana*, experimental work is needed to identify possible cryptic variation in host use, that is, whether a nominal species is actually subdivided into a complex of populations or sibling species with divergent host ranges. Thus, determining host range requires determining the kind of biological variation one is studying. For herbivores that have not been studied in this comprehensive manner, the potential for ambiguity might be usefully highlighted by speaking of a “nominal” host range. Acknowledging such ambiguity is critical as host range bears not only on ecological speciation but also on the evolution of ecological specialization, the macroevolutionary dynamics of host use, crop pest control strategies, and so forth (e.g., [32, 33]).

Note that these terms and issues may analogously be applied to the host range of nonherbivore parasites or to the “ecological range” of nonparasitic taxa.

5. “Host Form”: A Missing Term in the Vocabulary of Biological Variation

Just as it is important to recognize when one has an incomplete understanding of host range, it is vital to assess confidence in one’s assessment of the kind of biological variation being addressed and to acknowledge the lack of same. Presuming the kind of variation one is dealing with without sufficient evidence to rule out alternatives risks an erroneous diagnosis while compromising inferences premised on the kind of variation at hand. For example, if different enantiomers are types instead presumed to be host races or separate species, associated conclusions about speciation processes will be erroneous. As in the case of host range, a problem that promotes such misinterpretations is a current vocabulary that is insufficient to describe uncertainties. And a lack of terms indicating ambiguity may encourage workers to be less circumspect and make a best guess as to the kind of variation at hand in the absence of sufficient evidence. As suggested for host range, one could simply qualify uncertain assignments as “nominal.” A more conservative approach might, however, be advantageous, especially in the frequent situations where variation along host plant lines has been documented, but current evidence is limited and remains consistent with multiple kinds of variation. The term “biotype” used to be rather haphazardly applied to diverse kinds of variation [10]. But this owed to a lack of consensus on the meaning of that term rather than to a conscious effort to indicate uncertainty. In recent years, workers have begun writing about “host-associated differentiation (HAD),” a more general and somewhat variably applied term recently defined by Dickey and Medina [34] as, “the formation of genetically divergent host-associated sub-populations.” However, this term is not broad enough to span all kinds of biological variation and describes the relationship between populations rather than the populations themselves.

It seems then that the field is missing what would be a valuable term, one that allowed workers to acknowledge the existence of host-associated variation, while remaining neutral on the kind of variation it represents. The creation of such a term would eliminate the necessity of guesswork while recognizing a phenomenon of interest and pointing it out as needing additional study.

Thus, *host forms* = groups of individuals or populations exhibiting host-associated biological variation, but where the kind of variation has not yet been diagnosed.

I am formally introducing this term for the first time here. That fact notwithstanding, I have used it in prior publications when referring to the populations of *N. bebbiana* leaf beetles associated with their six different host plants [11, 29, 31, 35–42]. As my prior use of “host form” in papers and talks has not led to its broader adoption by the field, I hope that this paper will more effectively encourage such usage (!). The coining of this term was motivated by three things: first, the demonstration of cryptic host-associated variation within *N. bebbiana*, namely, patterns of relative acceptance of the six *N. bebbiana* hosts that varied according to the native host of the test population [31]. Second, insufficient data to infer what kind of biological variation was represented by beetles natively associated with each of the six hosts. Third, the lack of an existing term suitable for describing this real yet underdetermined kind of host-associated variation. Thus, I coined “host form” as an evolutionarily neutral and needed missing term in the vocabulary of biological variation. I have also introduced “ecological form” as an analogously neutral term to be applied to underinvestigated nonparasitic taxa exhibiting some form of ecological differentiation.
6. Host Races: Ever Intriguing, Overdiagnosed

Among the different kinds of biological variation, host races might be argued to be the most intriguing, at least to students of speciation. The reasons are twofold (see [21, 26, 43], for discussion of the following issues). First, host races represent intermediate points along the speciation continuum and thus provide unusual opportunities for studying speciation as a process. This is because host races exhibit considerable but incomplete reproductive isolation. This reproductive isolation, in concert with divergent selection, can facilitate or maintain ecological differentiation between host races and genomic differentiation at regions under sufficiently strong divergent selection. The gene flow that does occur prevents the fixation of alternative alleles even at selected loci, while homogenizing the majority of the genome between populations(see, e.g., [44]). However, whether host races will continue along the path to speciation, remain indefinitely at an equilibrium level of differentiation, or ultimately collapse back into a single population is never known.

Second, because host races demonstrate that adaptive differentiation and partial reproductive isolation can be maintained in the face of local gene flow, they have long been offered as evidence for the plausibility of sympatric speciation (e.g., [9]). This is important because, as noted earlier, the theoretical conditions under which sympatric speciation can occur are generally restrictive. However, although the existence of host races is consistent with sympatric speciation, it does not imply sympatric speciation. Indeed, the most theoretically onerous stages of sympatric speciation are the early ones in which reproductive isolation must evolve from within an initially panmictic population [14]. Thus, unless sympatric origins have been demonstrated, host races may alternatively reflect secondary contact and the maintenance of differentiation between populations whose initial divergence occurred allopatrically. Nonetheless, an erroneous bond between these terms can be found in the literature whereby claims of host race status are implicitly or explicitly linked with an inference of sympatric speciation. This bond may have promoted an overestimation of how amply sympatric speciation has been documented. This is somewhat similar to how the overlap between ecological speciation and sympatric speciation has led to inappropriate equating of these concepts and an overestimate of the latter.

To take this argument back a step, a greater problem is a considerable overdiagnosis of host races themselves that runs boldly through the literature. This despite a long history of delineating the criteria for host race status [10, 13, 45], culminating in an especially well-detailed and argued characterization of these criteria [26]. According to Drés and Mallet, host races are sets of populations with the following characteristics. (1a) They use different host taxa in the wild and (1b) consist of individuals that exhibit host fidelity to their respective hosts. (2) They coexist in sympatry in at least part of their range. (3a) They are genetically differentiated at more than one locus and (3b) are more genetically differentiated from each other in sympatry than either is from some geographically distant populations on the same host. (4a) They display a correlation between host choice and (4b) undergo appreciable gene flow. (5a) They have higher fitness on natal than alternative hosts and (5b) produce hybrids that are less fit than parental forms. (Note that analogous criteria must be met for populations of nonherbivorous parasites to be appropriately assigned host race status or for nonparasites to be assigned sympatric race status.) The authors apply these criteria to the literature in search of study systems that qualify as host races. Notably, this search turned up only three such systems, and even these are listed as uncertain for criterion (5b). This number is strikingly low. And even if the ten years since this paper’s publication has seen the documentation of more such cases, it seems unlikely that compelling evidence for host races is nearly as common as their invocation in the literature would imply. Surely this term is being misapplied. Why so?

Three related reasons come to mind. First, consider again that sympatric speciation is theoretically onerous and thus often argued to be rare and also that understanding patterns of diversification (e.g., the spatial coexistence of related species) would be much simpler should this speciation mode be plausibly frequent. For these reasons, evidence supporting sympatric speciation is rightly greeted with special excitement. Consider again too that evidence for host races is sometimes considered de facto evidence for sympatric speciation. Due to this connection, then, work claiming host race documentation might also be viewed as especially noteworthy, above and beyond the intrigue rightly accorded to host races per se. Because journals seek to publish important and broadly interesting findings, a selection process may thus be inadvertently imposed that favors the publication of such work and thus the making of such claims. Somewhat conversely, a second reason may be that as the plausibility of sympatric speciation has become more widely accepted in recent years ([46], e.g., announces its comeback) so has the plausibility of host races, again by virtue of their perceived equivalence. Presuming the veracity of this view, there may be every good reason, from the perspective of likelihood, to suspect that a case of host-associated differentiation could potentially be a case of host race formation. Thus, data consistent with, if not specifically demonstrative of, host races may more likely be used as the basis for a paper focusing on hopeful host races. The third reason is based on what sometimes appears to be the invocation of host races without any apparent justification. Here, it might be suspected that workers have simply not familiarized themselves with the criteria for diagnosing host races and that their frequent invocation has, via a positive feedback loop, resulted in such haphazard use as to make this term almost synonymous with host-associated variation per se. That is, it appears that in some circles this most intriguing and empirically onerous term is now serving the purpose of the least restrictive of terms, that of the here-proposed “host form.” This explanation might account for my own experiences of having N. bebbiana populations that I have consistently referred to as “host forms” nevertheless described in paper and grant reviews as “host races.” Hopefully, the introduction of host form into the vocabulary of biological variation will help restore host race to its proper place as an inherently fascinating phenomenon and a more reluctantly invoked term.
7. From Host Forms to Host Races: The Maple- and Willow-Associated Populations of *N. bebbiana*

Readers to this point may suspect that I hold views antithetical to the notions of host races and sympatric speciation. Rather, I am fairly agnostic on the issue of how evolutionarily frequent and important these phenomena are. Indeed, recent population genomic work may herald the common documentation of adaptive genomic differentiation in the face of gene flow [42]. However, just as Drés and Mallet [26] have provided criteria for documenting host races that seldom seem to have been demonstrated, so too does sympatric speciation theory lay out predicted parameters that have rarely been estimated [15]. Part of the problem is undoubtedly that obtaining adequate data for any one system requires a great amount of work. And sealing a strong case for sympatric speciation requires information—on the historical distributions of populations/species—that is rarely available. The latter point may forever prohibit claims about sympatric speciation (pro or con) in my focal study system, *Neochlamisus* leaf beetles. However, work on two of the six *N. bebbiana* host forms, those associated with maple and willow, has by now given me sufficient confidence to cease my use of “host forms” to describe these populations and declare, here, that they represent proper host races. Following the criteria of Drés and Mallet [26], the findings on which this claim is based are as follows.

(1a) These host forms are associated with divergent host taxa in nature, using red maple (*Acer rubrum*, Aceraceae) and Bebb’s willow (*Salix bebbiana*, Salicaceae), respectively [35].

(1b) Choice experiments involving host cuttings in habitat and leaf fragments in Petri dishes demonstrate that each sex of both host forms spends the majority of its time on its native host, demonstrating host fidelity [35].

(2) These host forms coexist in sympatry and syntopy in the same disturbed and riparian habitats across a broad region of northeastern North America (New England, New York, and southeastern Canada), where they commonly grow intermixed with each other. For example, I have documented host-associated differentiation in sympatric populations from Cumberland Co., ME, Kennebec Co., ME, Middlesex Co., MA, Exeter Co., NH, Oswego Co., NY (from 2 different sites), and Caledonia Co., VT.

(3a) Genome scan data reveal multiple loci that are more highly differentiated than expected under drift [44]. Many of these putatively divergently selected “outlier” loci are only differentiated in comparisons of populations using different hosts, implicating them in divergent host adaptation. Notably, this differentiation is observed between sympatric populations of the host forms from a site in northern Vermont.

(3b) Population trees based on these host-associated outlier loci show these sympatric host forms to be more differentiated from each other than either is to allopatric populations of the same host form [44].

(4a) The host fidelity of these host forms is associated with mating fidelity, in that positively assortative mating (= sexual isolation) is observed between them, with each one mating more readily with individuals of the same host form [11, 35].

(4b) Population trees based on putatively neutral loci group the sympatric populations together as more genetically similar to each other than either is to allopatric populations of the same host form, as expected if gene flow has homogenized neutral loci in sympathy [44].

(5a) Each host form grows faster and survives better on its native host than on the host of the other host form [35, 41, 47].

(5b) Hybrids between these host forms exhibit reduced growth rate and survivorship than the parental types do on their native hosts [41, 47].

*N. bebbiana* thus presents one of the very few examples for which data meet all the criteria of host races. They also more generally represent an increasingly well-understood case of potentially ongoing ecological speciation.

8. Conclusion

As does any form of rigorous inquiry, the accurate, informed study of ecological speciation depends crucially on terminological issues. It requires that terms be used in a manner consistent with their definitions. It requires that terms be qualified as necessary to accurately convey degree of confidence. It requires that “missing” terms be created as needed to fill conceptual gaps. It requires that inappropriate synonymizing, loose usage, and simple terminological ignorance be avoided. As argued here, deviations from these prescriptions seem to have led to overestimation of the evidence supporting host races and sympatric speciation, and the diffusion of the former term’s special status as an important evolutionary phenomenon. This loss may owe to the prior lack of an alternative term to convey the existence of host-differentiated entities whose evolutionary status cannot yet be determined. Such a term, “host form,” is proposed here as an important conceptual gap-filler. By contrast, the proper application of the term host race is illustrated here by describing how each of its criteria are fulfilled by maple- and willow-associated populations of *N. bebbiana* leaf beetles. Although the focus of this paper has been on herbivorous insects, the appropriate application of (sometimes new) terms and concepts across diverse taxa is also highlighted.

References


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Learning the Hard Way: Imprinting Can Enhance Enforced Shifts in Habitat Choice

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We investigated the potential importance of learning in habitat choice within a young hybrid zone of two closely related species of birds. Pied flycatchers (Ficedula hypoleuca) are being excluded from deciduous habitats into a mixed forest type by collared flycatchers (F. albicollis). We investigated whether this enforced habitat shift influenced reproductive isolation between the two species, and, by cross-fostering nestlings, we tested whether learning may lead to a corresponding shift in habitat choice in consecutive generations. Our results show that the majority of the recruits, even if translocated across different habitat types, return to breed in the area where they were fostered. As male pied flycatchers were more likely to hybridize in the originally preferred habitat, we argue that early imprinting on an alternate habitat can play an important role in increasing reproductive isolation and facilitate regional coexistence between species experiencing secondary contact.

1. Introduction

In the past decade, a revived interest in the role of adaptation to different environments in speciation, that is, ecological speciation, has emerged [1–4]. One important mechanism underlying ecological speciation is the buildup of reproductive isolation caused by habitat segregation (i.e., habitat isolation, e.g., [5]). Theoretical models of sympatric speciation generally include disruptive selection in resource use, which by extension can lead to differences in habitat choice and thereby reduced interbreeding [6–12]. Adaptive differences in habitat use can moreover evolve among allopatric populations, resulting in habitat isolation at secondary contact [13, 14]. Habitat isolation between species can also evolve at secondary contact, where reinforcement and competition are plausible drivers [15].

One factor suggested to potentially both promote and reduce the likelihood of ecological speciation is phenotypic plasticity [16]. Phenotypic plasticity can be defined as changes in an individual's behavior, morphology, and physiology directly induced by different environmental conditions [17–19] and is adaptive if individuals showing a plastic response have higher fitness than those that do not. In particular, moderate levels of plasticity appear to enhance evolution in novel environments by placing populations under directional selection towards new adaptive peaks [17]. Thibert-Plante and Hendry [16] found that plasticity also could counteract the buildup of reproductive isolation between populations after colonization of new environments. This is because selection against immigrants can be mitigated if dispersal occurs before the plastic adjustment to the environment. Thus, plasticity may have a large impact on the speciation process. Still, an underlying assumption in many models on the evolution of habitat isolation is that habitat choice is a genetically determined trait. However, learned habitat choice occurs in several animal species [20] and may, together with other forms of learning (e.g., sexual imprinting; [21]), be of importance in relation to speciation [6, 16, 18, 22, 23]. Beltman and Metz [24] found that speciation is more likely to occur through a learned than through a genetic habitat preference, unless the cost of learning is high, which, together with recent empirical findings (reviewed by [20, 25]), indicates that the relevance of learning in speciation may have been underestimated.
In this study, we use a combination of long-term breeding data and an experimental approach to investigate mechanisms behind divergence in habitat choice within a young hybrid zone of two closely related, hybridizing, and competing species of *Ficedula* flycatchers. Collared (*Ficedula albicollis*) and pied (*F. hypoleuca*) flycatchers cooccur in central and eastern Europe and on the Baltic isles of Öland and Gotland in Sweden [26]. The two species started to diverge 1-2 million years ago and were probably periodically isolated in separate glacial refuges during the Pleistocene, before expanding their breeding ranges northward [27]. Both species are migratory and winter in Africa in separated, but probably slightly overlapping, areas [28]. Pied flycatchers migrate along the Iberian Peninsula and winter in western to central Africa, and collared flycatchers winter in southeastern Africa, taking a more eastern migration route through Italy or further east [28, 29]. Males of the two species compete fiercely over natural tree holes or nest boxes after arrival at the breeding grounds in Europe [30, 31], and the two species overlap in timing of breeding [32] and feeding habits [33] suggesting strong interspecific competition. Collared flycatcher offspring have a higher growth potential under favorable environmental conditions [32, 34], but pied flycatchers are relatively more robust to harsh environments and towards the seasonal decline in food availability, providing them an opportunity to prolong coexistence with the otherwise more aggressive collared flycatchers [32, 34]. Pied flycatchers prefer to breed in deciduous forests [29] but are often found in coniferous forests on Öland [35]. The proportion of deciduous trees in pied flycatcher territories has declined in recent years as the number of collared flycatchers has increased [36] suggesting that competition-mediated habitat segregation is currently taking place. Here, we investigate potential mechanisms enhancing divergent habitat choice in these two ecologically similar *Ficedula* flycatcher species. More specifically, we test the role of learning in maintaining initially enforced shifts in habitat choice.

### 2. Material and Methods

#### 2.1. Study System

Collared flycatchers started to colonize Öland, where pied flycatchers were already present, in the late 1950s–early 1960s, and since then the relative proportion of pied flycatchers has quickly declined in the most favorable breeding sites [32]. There is species assortative mating in the mixed study population on Öland, but 5% of all pairs are heterospecific, and approximately 2–7% of the breeding flycatchers in different mixed populations are hybrids (reviewed in [26]). Male hybrids have lowered fitness, mainly due to disadvantages in competition over mates, while female hybrids are sterile in accordance with Haldane’s rule [37]. On Öland, the two species breed in 21 nest-box areas situated across the island (Figure 1). The landscape on Öland is characterized by a limestone plain covered by a thin soil layer. The southern part of the island is dominated by agricultural land, the middle part by deciduous forest, and the very north of the island contains mixed or coniferous dominated forests (Figure 1). Females lay one egg each day, and every nest box is monitored every third day to assess the starting day of egg laying. All breeding birds and their offspring are marked individually with numbered metal rings, and a small amount of blood is sampled for genetic analyses. Yearly records are kept on onset of egg laying, clutch size, hatching date, fledging, and fledging success. Morphological characters are measured in a standardized manner [30]. Adult females are caught when incubating the eggs, and adult males are caught when feeding the nestlings. Nestlings are weighed at day 7 and 13, and tarsus length is measured at day 13, a couple of days before fledging. Monitoring of the nest boxes has been performed during the periods 1981–1985 and from 2002 onwards. The breeding habitat was measured as the abundance of each tree species 360° around the nest boxes using a “relascope” (see [35]), which is a scale held at a fixed distance from the eye. By looking through an opening in the scale, individual trees are assigned into three categories based on trunk size and distance from the nest box. To investigate whether the habitat choice of pied flycatchers influences the risk of hybridization, we compared the habitat composition of con- and heterospecifically paired male pied flycatchers.

![Figure 1: Landscape types (1 = mixed or coniferous forest, 2 = deciduous forest, 3 = agricultural land) and distribution of *Ficedula* flycatcher nest-box areas (blue circles) on the Swedish island of Öland. The large circle represents nine nest-box areas surrounding the town of Löttorp where collared flycatchers started to colonize the island (and where pied flycatchers were already present) in the late 1950s–early 1960s. Collared flycatchers now outnumber pied flycatchers in this area (~90% collared flycatchers). The relative proportion of collared flycatchers gradually declines southward. Two of the nest-box areas in the north of the island are dominated by coniferous forest and so far only inhabited by pied flycatchers.](image)
Figure 2: Cross-fostering of nestlings between collared (CF) and pied flycatchers (PF) on the Swedish island of Öland using two different approaches. (a) Complete clutches of eggs swapped between nests in three steps. (b) Partial cross-fostering of three day old nestlings between two nests. The left side (i) refers to the nests before swapping, and the right side (ii) refers to the same nests after swapping (collared flycatchers in white and pied flycatchers in grey).

2.2. Cross-Fostering Experiment. We investigated the role of early learning in breeding habitat choice by comparing patterns of recruitment of birds that had been reared by foster parents. To increase the likelihood of recruiting nestlings reared in a new environment, we swapped complete clutches of eggs three days before the expected hatching date between different nests; the genetic parents nest (i.e., the nest of origin) and the foster parents nest (i.e., the nest of fledging). The cross-fostering experiment was performed between three nests with the same clutch size and date of egg laying; from collared flycatcher to collared flycatcher (to be used as a control in another study; Vallin et al. unpublished manuscript), from collared flycatcher to pied flycatcher with the pied flycatcher eggs thereafter being translocated back to the original collared flycatcher nest (Figure 2(a)). In addition, we also included recruits stemming from earlier cross-fostering experiments in our analysis. In these cross-fostering experiments, broods with coinciding hatching dates were split in half and nestlings were swapped between nests at the age of three days (e.g., [34, 38], Figure 2(b)). In one study [32], cross-fostering was performed with an additional brood size manipulation to simulate good and harsh environments. All nestlings were individually marked by clipping their toenails to enable individual measurements of growth and to ensure correct identification of species and nest of origin. JMP 8 (SAS Institute, Cary, NC, USA) was used to analyze the data.

3. Results

Pied flycatchers are currently being excluded from the preferred deciduous habitats on Öland, and we compared how the general habitat composition (proportion of deciduous trees) influenced the chance of pairing with a con- or heterospecific female among male pied flycatchers using a generalized linear model adjusted for overdispersion with binomial errors and a logit link. We found that hybridizing male pied flycatchers had a significantly higher proportion of deciduous trees in their breeding territories than males paired to a conspecific female ($N = 202, df = 1, \chi^2 = 14.19, P < 0.001$, Figure 3).

We used cross fostering experiments to investigate whether learning may influence habitat choice. In total, we included 264 nests subject to cross-fostering between the years 2002–2009 in these analyses: 96 nests where eggs were translocated and 168 nests where nestlings were cross-fostered. Many of the study plots are located far apart (maximum distance ∼85000 m, Figure 1) with pronounced differences in habitat composition [32]. We compared the general habitat composition (proportion of deciduous trees) among the pied and collared flycatcher territories that nestlings were swapped between using a generalized linear model adjusted for overdispersion with binomial errors and a logit link. We found that the habitat composition of pied and collared flycatchers included in the experiment was significantly different ($N = 48, \chi^2 = 10.62, df = 1, P = 0.001$), with a higher proportion of coniferous trees in pied flycatcher territories (Figure 4). Thus, in general nestlings that were swapped into a new nest experienced a different rearing environment than they would have done if they had been reared by their genetic parents.

We identified 49 recruits stemming from the experimental nests. The average recruitment rate was 4.3% (40 recruits
from 933 confirmed fledged offspring, i.e., data on fledging success were missing from the nests of 9 recruits). There was no significant difference in recruitment between nests depending on whether eggs or nestlings were swapped ($N = 218$, $\chi^2 = 0.704$, $df = 1$, $P = 0.40$), and no significant difference in recruitment between the species ($N = 218$, $\chi^2 = 0.386$, $df = 1$, $P = 0.53$); pied flycatchers recruited 21 juveniles from a total of 431 confirmed fledged offspring and collared flycatchers recruited 19 juveniles from a total of 502 confirmed fledged offspring. Of the 49 recruits from the cross-fostering experiments, 30 (61.2%) were recruits that had been swapped into a different rearing nest than the one attended by their genetic parents, and 24 of those were recruits from swaps between pied and collared flycatchers: 20 collared flycatchers (11 males and 9 females) and 4 pied flycatchers (1 male and 3 females). Thus, collared flycatchers had a significantly higher chance of recruiting back to the population compared to pied flycatchers when being raised by foster parents (two-tailed Fisher’s exact test: $N = 24$, $P = 0.03$).

We applied a matched pairs $t$-test to test whether nestlings cross-fostered between pied and collared flycatchers returned closer to the breeding site of their genetic parents or to the breeding site of their foster parents. GPS coordinates (Swedish grid system RT90) of the nest-box locations were taken and the Euclidean distance in meters between them calculated using Pythagoras’ formula.

The distance between the nest where the recruits returned to breed and the nest of the foster parents was significantly shorter than the distance between the nest where they bred as recruited and the nest of origin (mean difference = 21732 m, $N = 24$, $t = 3.92722$, $df = 23$, $P < 0.001$). The mean distance between the foster parents nest and the nest where the recruit subsequently settled was 1022 meters ($N = 24$, range = 33–4256 m, $se = 247$, $P = 0.009$, Figure 6(a)). Similarly, an analysis with a subset of collard flycatcher recruits that had been swapped between different nest-box areas revealed that also juvenile collared flycatchers shifted their breeding habitat choice when they had been reared in a different environment as compared to their genetic parents (two-tailed Fisher’s exact test, $N = 28$, $P = 0.0240$, Figure 6(b)).

### 4. Discussion

Pied flycatchers are currently being rapidly excluded from their preferred deciduous habitats on Oland and are instead increasingly found breeding in mixed or coniferous forests [32, 36]. We tested if the breeding habitat of male pied flycatchers influenced the risk of hybridizing and found that male pied flycatchers were more likely to pair with a heterospecific female in deciduous habitats, that is, the observed shift in habitat occupancy increases premating isolation between the species. We investigated whether learning may speed up segregation in habitat choice. By cross-fostering nestling Ficedula flycatchers, we found that the recruits, that is, one-year old birds returning to breed themselves, settled closer to the territory of their foster parents than to the territory of their genetic parents (Figure 5). In fact, the majority of the recruits, even if translocated into a different habitat type, returned as adults to breed in the same woodlot as they were fostered into. A similar pattern was found for both species but the comparatively low proportion of pied flycatchers returning to breed as adults after being raised by foster parents (4 pied flycatchers versus 20 collared flycatchers) means that we cannot investigate if there is a difference in the strength of learned habitat choice between the two species. The difference in recruitment per se is likely...
Figure 6: Habitat composition in the breeding territories of cross-fostered yearling pied (a) and collared flycatchers (b) as compared to the breeding territories of their foster parents and genetic parents on Öland, Sweden, 2002–2009. Both pied and collared flycatcher recruits returned to breed in a habitat different from their genetic parents. Only recruits that were swapped between different nest-box areas are included in this comparison, sample sizes are given above the bars.

Figure 6: Habitat composition in the breeding territories of cross-fostered yearling pied (a) and collared flycatchers (b) as compared to the breeding territories of their foster parents and genetic parents on Öland, Sweden, 2002–2009. Both pied and collared flycatcher recruits returned to breed in a habitat different from their genetic parents. Only recruits that were swapped between different nest-box areas are included in this comparison, sample sizes are given above the bars.

to reflect the fact that pied flycatchers are having problems establishing territories in areas inhabited by collared flycatchers [39]. Another potential source contributing to the biased pattern of recruitment might be that collared flycatcher nestlings sharing nests with pied flycatcher nestlings have a competitive advantage in achieving food from the parents [38], which could increase the likelihood of reaching a threshold weight for successful recruitment (e.g., [29]).

Several studies indicate a poorer quality in terms of availability of the food that flycatchers feed their nestlings with in coniferous habitats as compared to deciduous habitats [35, 40, 41], and allopatric populations of pied flycatchers experience higher reproductive success in deciduous habitats [29, 42]. Coniferous habitats are therefore unlikely to be the originally preferred habitat of pied flycatchers and in the young hybrid zone on Öland competition with collared flycatchers appears to be responsible for a rapid shift in habitat occupancy [36]. Our present study provides a mechanism by which a competition-mediated switch into a suboptimal habitat could result in a changed habitat choice through learning. We found that being reared in the initially less preferred habitat even makes collared flycatchers return to breed in this habitat as young adults. By extension, the fact that young birds develop a preference for breeding in the same type of habitat as they were reared in means that an enforced habitat shift due to competition quickly can result in divergence in habitat choice and strengthen premating isolation.

One may argue that a possible alternative explanation to our findings is that flycatchers exhibit a genetically fixed strategy to return to breed within a certain distance from their parents’ nest. However, several previous findings are not compatible with this alternative view. On the Swedish mainland, pied flycatcher nestlings and their parents do not stay in close vicinity to the nest site once the offspring are fledged and the nestlings do not return to breed within the same areas as their parents [29]. On the islands of Öland and Gotland, such movements are more limited. Moreover, aviary experiments performed in the older hybrid zone in central Europe suggest that pied flycatchers have developed a preference to feed in coniferous trees [43], and these birds were caught in a coniferous habitat as the process of habitat segregation has reached a later stage in this older hybrid zone [44].

For how long do the fledglings stay in their natal area, and when does imprinting occur? Van Balen [45] studied postfledging dispersal in a Dutch pied flycatcher population and found that juvenile birds tend to stay within 600 m of their hatching site up to about 50 days. Berndt and Winkel [46] transferred both eggs and fledglings (about 36 days old) of pied flycatchers between areas located 250 km apart in northern Germany. They found that all returning birds came back to the area where they had been transferred, showing that imprinting can take place even after three weeks postfledging. A recent study on flycatchers reveals that it is possible to translocate also adult birds between environments [47]. Apart from opening up new possibilities to study the fitness consequences of breeding in different habitats, this finding also indicate that the importance of plasticity and learning in general might have been underestimated in studies of avian habitat choice. By showing that birds that have been experimentally reared in a different environment, as compared to their genetic parents, subsequently return to the area of their foster parents, our results provide additional support for the importance of early learning in habitat choice, also on a small geographical scale.

In a few cases, the birds recruiting from our experiment settled to breed relatively far away from their foster area. Although we cannot make any inferences from those few
cases, future studies could try to pinpoint detailed differences in dispersal strategies and habitat choice in relation to, for example, population density, relative frequency of competitors, and variation in microhabitat quality. Natal conditions are likely to affect dispersal propensity in a number of ways. For example, high-quality habitats may produce more individuals prone to disperse as compared to low-quality habitats [48]. Lens and Dhondt [49] found a link between habitat condition and natal dispersal in crested tits (Parus cristatus), where individuals from higher quality habitats dispersed more readily than individuals from lower-quality habitats. Heritable differences in dispersal propensity in coupling with other behaviors (e.g., aggression) have been suggested to be especially important during natural range expansions or hybrid zone movements in birds [50]. However, as Davis and Stamps [20] point out: if dispersers have a strong innate preference for their natal habitat type, the degree of similarity between patches will influence the rate of dispersal such that dispersal into novel habitats is prohibited. On the other hand, recent bird studies indicate a strong role for habitat-related cultural transmission through early learning, for example, in the feeding strategy of great tits [51], natal dispersal of pied flycatchers [52], and habitat selection in the warbler finches of Galápagos, where females choose to breed on islands with habitats similar to their natal environment [53].

Tonnis et al. [53] found a positive correlation between genetic distances in the warbler finches of Galápagos and differences in maximum elevation among islands and concluded that habitat selection may have helped to initiate the speciation process. The genetic component of habitat preferences might be larger in more specialized populations, and speciation may occur as assortative mating evolves [24]. The effectiveness of learned habitat preferences in speciation could be due to being a one-allele mechanism [54], whereas speciation through a genetic habitat preference has been suggested to be a two-allele mechanism [24]. In other words, when habitat choice is genetically determined, genetic recombination can lead to a breakdown of the associations between the alleles underlying habitat choice and the alleles underlying ecological adaptation, whereas this is not a problem with a learned habitat preference. Song learning is another example of a culturally transmitted trait in birds suggested to operate in a similar way [55].

In summary, we have shown that an enforced shift in breeding habitat choice can be further enhanced by early imprinting on the natal area. From a theoretical point of view, learned habitat choice might be a powerful mechanism of generating assortative mating [24]. When one species shifts breeding habitat due to interspecific competition, segregation in habitat choice will increase (and premating isolation as a side effect) when recruitment into the alternative environment exceeds recruitment into the originally preferred environment due to learning (i.e., imprinting on the natal environment). Thus, learned habitat choice can play an important role both in enhancing reproductive isolation and in facilitating regional coexistence at secondary contact between diverging populations.

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References


Review Article

Sympatric Speciation in Threespine Stickleback: Why Not?

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Numerous theoretical models suggest that sympatric speciation is possible when frequency-dependent interactions such as intraspecific competition drive disruptive selection on a trait that is also subject to assortative mating. Here, I review recent evidence that both conditions are met in lake populations of threespine stickleback (Gasterosteus aculeatus). Nonetheless, sympatric speciation appears to be rare or absent in stickleback. If stickleback qualitatively fit the theoretical requirements for sympatric speciation, why do they not undergo sympatric speciation? I present simulations showing that disruptive selection and assortative mating in stickleback, though present, are too weak to drive speciation. Furthermore, I summarize empirical evidence that disruptive selection in stickleback drives other forms of evolutionary diversification (plasticity, increased trait variance, and sexual dimorphism) instead of speciation. In conclusion, core assumptions of sympatric speciation theory seem to be qualitatively reasonable for stickleback, but speciation may nevertheless fail because of (i) quantitative mismatches with theory and (ii) alternative evolutionary outcomes.

1. Introduction

The feasibility and prevalence of sympatric speciation have been in contention since the birth of evolutionary biology [1, 2]. Recent empirical work on sympatric speciation has focused on documenting a few likely examples [3–6], or estimating the relative frequency of various geographic modes of speciation [7]. Concurrently, theory has focused on determining which conditions are necessary and sufficient for sympatric speciation to occur [8–11]. Unfortunately, these two related research programs rarely intersect. Namely, there is a need for models based on empirically measurable parameters, preferably tailored to the natural history of specific case studies (e.g., [12, 13]), and for empirical estimates of key parameters in such models. Such fusions of empirical data and theory will provide more biologically realistic insights into when or why sympatric speciation might succeed or fail, and thus explain its frequency.

In this paper, I attempt such a fusion, by comparing simulation results to empirically derived parameter estimates from threespine stickleback (Gasterosteus aculeatus). The goal is to understand why sympatric speciation is rare or absent in this organism [14] despite a qualitative fit with some key requirements for speciation. The objections to sympatric speciation are so well known [1, 2] that many biologists take it for granted that sympatric speciation is unlikely. If so, why bother explaining a specific example in which sympatric speciation fails? The answer is that we might find sympatric speciation fails for unexpected reasons.

2. Theoretical Background

Theory has revealed two key underpinnings of sympatric speciation. First, a negative frequency-dependent process such as intraspecific competition is required to generate persistent disruptive selection [15–18], which splits the population into phenotypically divergent groups. Second, assortative mating is required to generate reproductive isolation that maintains those divergent groups in the face of recombination [19, 20]. There is a broad consensus from existing theory that sympatric speciation is easiest when disruptive selection and assortative mating act on the same trait (or closely correlated traits) [11]. Such pleiotropic traits have been dubbed “magic traits” for their uniquely favorable role in speciation [21], because they prevent recombination from decoupling the target of mate choice and selection.
Figure 1: Illustration of the evidence for phenotypic and diet variation, phenotype-diet correlation, disruptive selection, and assortative mating in stickleback. (a) Phenotypic variation in gill raker length within a population of stickleback (First Lake, 2008; [22]). (b) Diet variation within a population of stickleback (First Lake, 2008; [22]) indicated by the high variance in carbon and nitrogen stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$ compared to the variance observed when individuals all consume the same set of prey (thick cross over the centroid represents ±2 s.d., based on lab-raised stickleback [23]). Adjusting for baseline isotopic variation (thin arrow indicates a benthic primary consumer [snails], thick arrow represents a planktivorous primary consumer [mussels]), individual stickleback fall anywhere from 0% to 100% benthic carbon. Because of the correlation between $\delta^{13}C$ and $\delta^{15}N$, the first isotopic principal component axis can be used to represent the axis from benthic to limnetic diets. (c) Phenotypic and diet variation are correlated: fish with longer gill rakers tend to consume a more limnetic diet (high values of isotopic PC1 indicate lower $\delta^{13}C$, higher $\delta^{15}N$; First Lake). (d) Assortative mating by diet, indicated by a positive correlation between males’ isotopic signature and the isotopes from eggs (indicative of female diet; modified from [23]). (e) A key measure of trophic morphology, gill raker length, is subject to disruptive selection as indicated by a significant quadratic coefficient in a regression of growth rate on size-standardized gill raker length. (First Lake, 2005; modified from [24]). Growth rate was measured using a biochemical index, the ratio of RNA to DNA in muscle tissue. The relationships in each panel of this figure are repeatable across multiple solitary lake populations.
Skeptics of sympatric speciation typically adopt several objections to this theory [1, 11, 20, 25], arguing that (i) models often assume excessively high initial genetic variance and/or high mutation rates, (ii) disruptive selection is likely to be dynamically unstable (populations evolve away from fitness minima towards regions of stabilizing selection), and therefore, disruptive selection should be rare, (iii) costs to mate choice will select against assortative mating, and (iv) “magic traits” subject to both selection and assortment are rare. Finally, casual readers of the literature on speciation theory often come away with the impression that sympatric speciation is all but inevitable when disruptive selection and assortative mating jointly act on a magic trait. In fact, speciation can only occur if these evolutionary forces are strong. Otherwise, the population may evolve higher trait variance, possibly even a bimodal phenotypic distribution with some assortative mating, but never reach full speciation in the sense of forming strongly reproductively isolated and phenotypically nonoverlapping populations [8]. Thus, the core theoretical conflict between skeptics and opponents of sympatric speciation concerns the biological realism of core assumptions, and thus can be best addressed by answering the following empirical questions.

(i) Do Single Populations Typically Exhibit Substantial Genetic Variance in Ecologically Relevant Traits and/or Mating Strategy? There is no doubt that many populations harbor substantial variation for ecologically relevant traits such as resource use. Within many populations, discrete morphotypes or quantitative trait variation give rise to heritable among-individual differences in prey preferences [26, 27]. As a result, individuals typically consume a small subset of their population’s collective diet (across 142 measures of diet variation from 35 species, there was an average of only 45% similarity between individual and population diets [28]). It is less clear how often populations harbor heritable variation for the degree of choosiness in assortative mating though such variation has been documented [29].

(ii) How Common Is Disruptive Selection? One can measure disruptive and stabilizing selection by quadratic regression of a fitness measure against a phenotype [30]. Positive quadratic coefficients (γ) imply disruptive selection if the fitness minimum lies within the range of extant phenotypes. A meta-analysis of selection gradients in nature found that positive and negative quadratic coefficients were about equally common and of similar magnitude (though both were weak: average |γ| ~ 0.1 [31]). This suggests that disruptive selection is more widespread than often believed, perhaps because it is stabilized by negative frequency-dependent interactions that prevent evolution away from fitness valleys towards fitness peaks [15, 32]. One caveat is that it is not known how many cases of positive curvature entail a real fitness minimum within the phenotypic range, as opposed to just a monotonic but curved fitness function.

(iii) How Common Is Assortative Mating within Populations? Assortative mating is the tendency for individuals to choose mates who are more (or less) phenotypically similar to themselves than expected by chance. The result is a phenotypic (or genetic) correlation between mated males and females, with respect to one or more traits expressed in both sexes. This correlation is easily measured by quantifying some trait in both sexes across multiple mated pairs. The magnitude and sign of this correlation provides a measure of the strength and direction of assortative mating. One caveat is that assortative mating might act on multiple traits concurrently, in which case multivariate canonical correlations might be more appropriate. Studies focusing on one or a few traits might thus underestimate assortative mating by overlooking one or more important traits. With these caveats in mind, a recent meta-analysis established that there is a broad tendency towards weak positive assortative mating within ostensibly panmictic populations (mean r = 0.23; [33]). Note that these positive correlations can arise from a variety of mate choice processes, including a preference for phenotypically similar mates (as assumed in many sympatric speciation models), spatial sorting of phenotypes at the time of mating, allochrony, or directional mate preferences in both sexes.

(iv) How often Do Disruptive Selection and Assortative Mating Affect the Same Trait? If disruptive selection and positive assortative mating are both moderately common, it is likely that they sometimes act concurrently in a given species, possibly on the same (or correlated) traits. Although examples of “magic traits” have been documented (particularly in incipient species pairs; e.g., [5, 34, 35]), we do not know their general frequency in nature, particularly within populations prior to speciation. Thus, at present, we must rely on insights obtained from specific case studies. For example, disruptive selection and assortative mating have been shown to act on the same traits in Darwin’s finches [36, 37] and in stickleback [23, 24]. In the remainder of this paper, I focus on the latter as a case study.

(v) When They Coincide, Are Disruptive Selection and Assortative Mating Strong Enough to Drive Speciation? To date, this question has remained essentially unanswered for two reasons. First, there are few species for which both processes are known to co-occur within a single population. Second, theoretical models are usually constructed using hard-to-measure parameters, making it hard to evaluate what constitutes (un)realistically strong parameter values. Three-spine stickleback (Gasterosteus aculeatus, a small north-temperate fish) is one of the few organisms known to experience both disruptive selection and assortative mating on a shared trait within single populations (see also studies of Darwin’s finches [36, 37]). In the remainder of this paper, I review recent work supporting this claim, focusing on processes occurring in single-species populations of stickleback that represent plausible precursors to speciation (Figure 1a); as opposed to the well known stickleback species pairs [38]). However, sympatric speciation is rare or absent in stickleback [14], raising the question, “Why has sympatric speciation failed to occur?” To answer this model,
I combine the reviewed empirical data with a new numerical simulation to show that disruptive selection and assortative mating in stickleback are too weak to drive speciation. Furthermore, I review empirical evidence that fluctuating selection and alternative forms of diversification further inhibit speciation. Thus, many of the common objections to sympatric speciation do not hold in this system, but other quantitative and qualitative hurdles remain, many of which are not widely incorporated into theoretical models.

3. Diversifying Forces within Lake Stickleback

3.1. Disruptive Selection. Theory predicts that stable disruptive selection can arise from frequency-dependent interactions such as intraspecific competition for heterogeneous resources [16]. This resource heterogeneity is most pronounced when the population uses two distinct resource types (e.g., benthic and limnetic prey [39], hard and soft seeds [36, 40], etc.) but can also apply to multimodal [41] or unimodally varying resources [42]. When co-occurring individuals use divergent subsets of the available resources, the level of competition experienced by a given individual depends on the density of like phenotypes that consume similar resources, rather than the total population density. Phenotypically average individuals experience stronger competition than rare extreme phenotypes, and thus suffer lower relative fitness (disruptive selection). Note that competition only involves the individual and the resulting disruptive selection.

Here, I focus on competitive disruptive selection, because this is the most widely modeled source of frequency dependence and the best documented in stickleback. For this disruptive selection to occur, a population must exhibit (i) high population density leading to resource limitation, (ii) among-individual variation in use of a diverse set of resources (known as “individual specialization”; [26]), and (iii) a measurable phenotype correlated with this resource use variation, which is thus subject to frequency-dependent competition. In the following paragraphs, I review the existing evidence from stickleback for each of these conditions and the resulting disruptive selection.

(i) Intraspecific Competition. Numerous laboratory studies confirm that food ration strongly affects stickleback growth, female egg production and spawning frequency, and male reproductive success [44–46]. Stickleback kept at approximately natural density in field enclosures reduced benthic invertebrate density by 54% [47], implying that stickleback exert top-down control of prey density as required for exploitative competition. Accordingly, experimentally elevated stickleback density in field enclosures suppressed prey density, reducing stickleback stomach content mass, growth, and reproductive investment [48, 49] and altered growth rates in seminatural ponds [50–52]. These results suggest that intraspecific competition has the potential to significantly impact stickleback. However, further work is required to provide direct proof of density-dependent population regulation in unmanipulated settings.

(ii) Individual Specialization. As a species, stickleback are ecological generalists, consuming a wide diversity of invertebrate prey including crustacean zooplankton, mites, aquatic and terrestrial insects, and even other stickleback. This is often characterized as a bimodal resource base, comprised large benthic macroinvertebrate prey and smaller limnetic zooplankton [38]. In reality, the resource base is more complex and can be described using a continuous distribution of prey body sizes: within solitary stickleback populations, individuals’ mean prey size conforms to a unimodal distribution [47]. Individuals also specialize more finely on prey taxa [41], and on microhabitat type [53], than the benthic/limnetic distinction would imply, though, the benthic/limnetic axis remains a convenient and fairly reasonable simplification.

Within a population, any given individual tends to be relatively specialized compared to its population as a whole [54]. For example, direct observation of foraging individuals in Little Mud Lake (Vancouver Island) showed that some individuals consistently directed more than 90% of their attacks at benthic prey, whereas others used 70% mid-water prey, or else specialized on surface prey [55]. Accordingly, within a lake stomach contents reveal among-individual diet variation ranging from mostly benthic to mostly limnetic prey [41]. This stomach content variation is corroborated by a longer-term diet measure based on stable isotope variation among individuals, which reveals that co-occurring individuals vary from 0% to 100% dietary benthic carbon. This isotopic variability is far greater than we expect when individuals feed on the same prey in the same proportions (Figure 1(b); [54]). We observe this diet heterogeneity even among individuals held in small (10 m^2) enclosures that ensure individuals have equal access to the same prey [56]. It is apparent that diet variation reflects among-individual differences in prey preferences and/or acquisition abilities, rather than coarse-grained differences in prey availability due to spatial separation of foraging individuals.

Some of the diet variation is attributed to morphological differences among individuals (Figure 1(c)). Within any given lake, individuals with relatively long gill rakers typically have more pelagic zooplankton in their stomachs and have correspondingly limnetic carbon and nitrogen stable isotope signatures [23, 54, 57–59]. Gill raker number, body size, body shape, jaw lever ratios, hyoid length, and buccal volume have also been found to be correlated with diet variation [41]. Importantly, several of these trophic traits have been shown to exhibit heritable variation [60–63], and thus should be capable of responding to selection.
(iii) Frequency Dependence. As a result of individual specialization, not all individuals within a population use the same resources. Within a typical population of stickleback any pair of individuals only share an average of 30% of their prey in common [41]. Because diet is correlated with morphology, this diet overlap is even lower between morphologically divergent individuals: in one lake, diet similarity was twice as high for the most morphologically similar individuals, compared with the most dissimilar individuals [54]. Consequently, the level of competition experienced by an individual depends not on total population density but on the abundance of individuals with overlapping diets (e.g., similar morphology). Intraspecific competition should thus favor individuals with rare feeding strategies over individuals with common strategies who have proportionally more competitors. Direct evidence for such frequency-dependent competition comes from experiments in artificial ponds. Schluter [52] manipulated the relative frequency of benthic and limnetic species pairs and their hybrids and found that a given phenotype experienced reduced growth when it was the more common form. Although the equivalent study has not yet been done within a single-species population of stickleback, it is reasonable to assume that similar processes operate. Within-population frequency dependence may of course be weaker, given the lower phenotypic variance compared with species-pair lakes [38].

The joint occurrence of competition and diet variation should drive frequency-dependent disruptive selection. Accordingly, a survey of multiple solitary populations of stickleback found a widespread tendency towards disruptive selection on two uncorrelated trophic traits (gill raker length and number; [24]). On average, phenotypically intermediate individuals (of either sex) grow more slowly (Figure 1(e)), attain smaller body size and invest proportionally less mass in reproductive tissue than individuals at either extreme of a given trait. The curvature of the estimated fitness landscape averaged $γ \approx 0.06$ (Figure 2), somewhat weaker than the average quadratic curvature found in Kingsolver et al.’s meta-analysis [31]. Artificially elevated stickleback density led to stronger-than-average disruptive selection, whereas reduced density eliminated disruptive selection altogether [48]. These results suggest that intraspecific competition generates disruptive selection in many lake populations of stickleback. However, an important caveat is that that none of the fitness metrics (adult growth rate and variation in reproductive tissue mass) represents total lifetime fitness. Consequently, although intermediate phenotypes grow more slowly as adults and invest less mass in gonads, it is possible that additional components of fitness (larval or juvenile growth, survival, mating success, breeding duration, etc.) might overwhelm the component of selection arising from foraging success as measured in the studies cited above. Because lifetime fitness is effectively impossible to track for individual stickleback of known morphology, our best available estimates of selection are likely to be incomplete.

Even taking these selection estimates at face value, disruptive selection is not ubiquitous. Large or small lakes are dominated by single habitat types (limnetic or benthic), whereas intermediate-sized lakes contain a more equal bination of these resources. Consequently, we expect disruptive selection to be more pronounced in intermediate-sized lakes. Indeed, disruptive selection seems to be maximized in intermediate-sized lakes between 40 to 60 ha surface area (there is a significant quadratic relationship between lake area and the quadratic selection coefficient $γ$ [24]). Disruptive selection is also modified by other ecological interactions. In experimental ponds, disruptive selection was exaggerated in the presence of predatory trout [50, 64]. The effect of interspecific competition is not well understood, but depending on the species, fish predators can increase or decrease the diet variation required for disruptive selection [65].

3.2. Assortative Mating. Positive assortative mating occurs when mated pairs are phenotypically more similar than expected by chance. There is abundant evidence that the benthic and limnetic species pairs exhibit premating reproductive isolation [66–70], as do some ecologically and phenotypically divergent allopatric populations [71, 72] though other divergent stickleback populations show no isolation [73]. Remarkably, assortative mating also occurs within solitary stickleback populations. Stable isotope analyses reveal that within a single population, males with more benthic isotope signatures had mated with more benthic females ($r = 0.507$; female diet inferred by isotope signature of their eggs; Figure 1(d); [23]). Unlike many studies of reproductive isolation between divergent populations, typically studied in laboratory aquaria, this intraspecific assortment is measured in the field with wild individuals, so it represents a more natural setting. The positive assortative mating represents a “magic trait” system, because individuals pair on the basis of a trait (isotope ratio) which is correlated with the ecomorphological traits under disruptive selection [23, 24]. In fact, individuals’ foraging strategy (reflected in isotopes)
may be the most direct target of selection, which results in correlated selection on trophic morphology [22].

At present, the mechanism underlying within-population assortative mating in stickleback is unknown. Assortative mating may arise indirectly if different phenotypes are spatially segregated during mating, as has been shown for the benthic and limnetic species pairs [70, 72]. Thus, microhabitat choice could contribute to within-population assortative mating. A spatially explicit follow-up study of isotopic assortative mating [74] (in a second lake population) confirmed the previous positive correlation [23]. Males and females were distributed nonrandomly in space; isotopically more limnetic individuals tended to nest deeper and closer to vegetation than benthic individuals (nests ranged from 0.5 to 2 m deep) [74]. However, isotopic assortative mating remained even after statistically controlling for spatial and habitat structure, implying a role for active mate preference.

Direct preferences for similar mates might involve morphological traits that are correlated with diet (e.g., size, body shape, and color) or cues derived directly from the prey. Some evidence is available for the latter effect: fish experimentally fed one food type for two weeks, subsequently exhibited a preference to shoal with conspecifics fed the same food [75]. This shoaling preference was maintained as long as the focal individuals received olfactory cues from the potential social groups. Similar results seem to hold when studying females’ mate preferences instead of just social affiliation [76]. Olfactory cues have also been implicated in MHC-based mate choice in stickleback [71, 77] though it is unclear to what extent this might interact with diet-induced assortment. Regardless of the exact mechanism underlying assortative pairing, the ultimate effect will be to maintain linkage disequilibrium among genes underlying ecologically diverging traits, thus facilitating speciation.

4. Does Sympatric Speciation Occur in Stickleback?

4.1. Rarity of Species Pairs. Despite disruptive selection and assortative mating acting on correlated traits, stickleback do not appear to undergo sympatric speciation with any great frequency, if at all [14]. Stickleback occur in north temperate coastal watersheds throughout the Atlantic and Pacific [78]. On Vancouver Island alone, they inhabit many hundreds of lakes that were all likely colonized shortly after deglaciation. Despite this plethora of similar-aged lake populations regionally and globally, only a handful of species pairs have been described. In British Columbia, species pairs have been documented in seven lakes (Balkwill, Enos, Hadley, Paxton, Priest, Emily, and Little Quarry Lakes) in five watersheds from four islands [38, 79, 80]. Collectively, researchers studying stickleback in the American and Canadian northwest have surveyed hundreds of lakes, suggesting that species pairs occur at most in a few percent of inhabited lakes. That said, it is likely that additional species pairs remain to be discovered: a new pair was described as recently as 2008 [79]. Also, it is possible that species pair lakes arise more frequently but commonly collapse [81, 82]. Such a taxon cycle seems unlikely, as the examples of recent species pair collapse entail anthropogenic environmental change [82, 83].

A few additional pairs of lacustrine sympatric “morphs” have been described outside of British Columbia [80]. In Alaska, Benka Lake stickleback caught in different habitats are morphologically divergent [84]. However, these ecotypes do not exhibit the bimodal trait distribution seen in species pair lakes, nor is it known whether they are genetically distinct populations. Similar divergence patterns are observed between substrate habitats within several lakes in Iceland [85]. This divergence can evolve rapidly: damming of a fjord in Iceland in 1987 led to rapid divergence from the marine ancestor, and differentiation among habitats [86]. However, these ecomorph pairs do not yet exhibit the near-complete reproductive isolation and genetic divergence that typified the British Columbian species pairs.

4.2. How Did the Few Species Pairs Arise? Not only are species pairs rare, but there is reason to doubt they arose via sympatric speciation. Two alternative models have been proposed for the geography of speciation in British Columbian species pairs [87, 88]. The first invokes standard sympatric speciation: marine stickleback colonized the postglacial lakes ~12,500 years ago, after which increased lake elevation due to isostatic rebound prevented recurrent colonization [87]. Subsequently, disruptive selection may have driven ecological divergence, which in turn led to reproductive isolation based on size, color, and behavior. Reproductive isolation may have been a pleiotropic effect of trait divergence [66, 68, 89], supplemented by reinforcement [69].

The alternative model invokes a brief period of allopatry [87]. The initial colonization by marine fish led to rapid adaptation into a generalist that may have resembled today’s solitary populations. A subsequent brief sea level rise [90] was thought to have introduced a second population of marine stickleback, which underwent ecological and reproductive character displacement with the native generalist form to produce, respectively, a limnetic and benthic species. This model is neither purely allopatric nor sympatric, since some component of reproductive isolation evolved in both contexts at different times. However, the critical initial step of divergence was allopatric.

For a brief period in the 1990s, phylogeographic data based on mitochondrial DNA sequences lent support to the sympatric model of stickleback speciation, because species pairs for each lake were typically reciprocally monophyletic [88]. However, subsequent analysis of microsatellites told a different story: each ecotype was most closely related to marine forms or to morphs in other lakes [91] though phylogenetic resolution remained poor. The monophyly of mtDNA sequences may thus be the result of introgression following secondary contact. Additional evidence for the allopatric model comes from the fact that limnetic stickleback are more tolerant of salinity [92] and genetically more related to marine forms than are benthic stickleback [91]. Also, all the species pair lakes are geographically nearby and at similar low elevation, suggesting that some
unique localized geological event facilitated speciation. Consequently, the case for an allopatric phase of divergence seems fairly strong. However, newer geological data does not support the required secondary sea level rise [93], making it hard to explain how a secondary introduction could have happened. Thus, biological data remains most consistent with the secondary contact scenario though the mechanism of secondary colonization is unclear (Schluter, pers. comm.). The question may be unresolvable unless perhaps ancient DNA analyses of subfossil stickleback bones in sediment could reveal evidence for a double invasion.

5. It Is Not That Easy: Simulations with Empirical Parameters

The absence (or at best, rarity) of sympatric speciation in lake stickleback despite disruptive selection and assortative mating raises the question, “why do stickleback apparently not experience sympatric speciation?” Several of the standard theoretical objections to sympatric speciation (Section 2, above) do not apply, as there is substantial polymorphism in resource use, disruptive selection, and assortative mating on the ecological trait, as described above. The simplest remaining hypothesis is that disruptive selection and/or assortment, while present, are too weak to complete speciation [8, 94, 95], or that some additional evolutionary processes impose additional constraints on reproductive isolation.

To test whether the observed selection and assortment are sufficient to drive speciation in a best case scenario, I performed stochastic numerical simulations of the joint effect of disruptive selection and assortative mating acting on a quantitative trait based on the additive effects of numerous small-effect loci (see the Appendix for model details). Unlike most previous models of sympatric speciation, the model used here focused on the effect of empirically measurable parameters (but see the empirically driven papers by Gavrilets and colleagues [12, 13]). Here, I focus on asking whether the quadratic selection gradients (γ) and the phenotypic correlations between mates (r) measured in stickleback are sufficient to split the phenotypically unimodal population into a bimodal distribution of two reproductively isolated populations. This focus on present-day parameter values sets aside the question of whether disruptive selection would drive increased assortative mating in the future. The reinforcement-like process often examined in sympatric speciation models [8, 9, 11, 96] remains empirically untested, and so, it is not studied here explicitly.

Replicate simulations were run for a range of empirically plausible values of disruptive selection and assortative mating to determine what parameter combinations are required for speciation. Speciation was said to have occurred if the initially unimodal phenotype distribution became strongly bimodal, indicated by two major peaks separated by a trough less than 5% as abundant as the lowest mode. The empirical measures from stickleback (reviewed above) were compared with the simulation results to determine whether solitary stickleback populations could plausibly be expected to speciate sympatrically (e.g., fell within the parameter space conducive to speciation). Note that my simulations focused on just these parameters and omits extensive consideration of other potentially relevant factors such as the genetic architecture of the trait, population size, mutation rate, and costs to choosiness.

Consistent with many previous theoretical models and simulations [97], I found that sympatric speciation was possible given the joint action of strong disruptive selection and assortative mating (Figure 3). By “strong” I mean quadratic selection coefficients of γ > 0.25; empirically, γ follows a double exponential distribution and ranges between about −1 to 1, with most values between −0.1 to 0.1 [31]. Speciation required strong positive assortative mating, defined here as a trait correlation above about 0.6; empirically r ranges from below −0.9 to above 0.9, with a mean of 0.23 [33]. However, if either evolutionary force is weak or moderate (e.g., average values of γ ~ 0.1 and r ~ 0.25), then speciation does not occur (Figure 4). Instead, populations may either remain unimodal but with inflated variance (compare Figure 5(a) with 5(b)), or may attain a uniform or even bimodal distribution (Figure 5(c)) in which assortative mating is too weak to prevent the continual and frequent recreation of intermediate phenotypes [8, 94, 98] but maintains some linkage disequilibrium that substantially inflates trait variance. Notably, however, sympatric speciation (Figure 5(d)) can be expected to occur with empirically reasonable (albeit strong) parameter values.

Empirically estimated values of disruptive selection and assortative mating in stickleback fall well within the parameter space where speciation is never observed (Figure 4). The strongest observed disruptive selection would be sufficient for speciation, if assortative mating were twice as strong as the observed average. The strongest observed assortative mating would be sufficient if disruptive selection were seven
times stronger than average (or more than twice the maximum). Even the joint effect of the empirical maximum disruptive selection and assortative mating would be insufficient for sympatric speciation (in simulations run for >50,000 generations). It is thus clear that solitary populations of stickleback are unlikely to undergo sympatric speciation in their current ecological setting. This is a reassuring result in that it may explain why solitary populations have remained solitary. Of course, this does not rule out the possibility that the few stickleback species pairs arose from populations with exceptionally high levels of one or both parameters.

One caveat regarding these simulation results is that I omit the possibility that assortative mating itself may evolve. Numerous models suggest that disruptive selection can drive a reinforcement-like process favoring the evolution of increased assortative mating [9, 43] though this possibility remains contentious [25, 99]. Thus, it is conceivable that stickleback populations experiencing the strongest levels of disruptive selection could evolve stronger mate preferences in the future. This increased choosiness depends on a variety of additional assumptions about the behavioral basis of assortative mating, the genetic architecture of mate choice, costs to mate choice, mating system, and so on. Lacking information about these parameters, it seems more appropriate at present to evaluate whether existing parameter values are sufficient to bifurcate populations, than to speculate over whether stickleback may or may not shift to elsewhere in parameter space. Although I do not explicitly model the evolution of assortment, it is possible to infer the result of a hypothetical increase in female choice, which amounts to moving horizontally right in the parameter space shown in Figure 4. For populations at the highest level of disruptive selection (Figure 4), such a rightward shift in parameter space could conceivably bring them into parameter space where

![Figure 4](image1.png)

**Figure 4**: Speciation is only observed when there is strong disruptive selection and strong assortative mating (shaded region). For comparison, the crossed lines indicates the range and mean (intersection) of these parameters measured in single-species lake populations of stickleback [23, 24]. Parameter values are population size = 500, number of loci = 40, and initial allele frequency = 0.5. Disruptive selection and assortative mating were varied systematically from $\gamma = 0$ to 0.5 and $r = 0$ to 0.95, in increments of 0.025. This parameter space falls well within empirically justifiable values based on meta-analyses [31, 33]. Ten replicate simulations were run for each factorial combination of parameters. Dark shading indicates regions of parameter space in which speciation was observed at least once.

![Figure 5](image2.png)

**Figure 5**: An illustration of various outcomes of disruptive selection and assortative mating, depending on parameter values. Each histogram represents the phenotype frequency distribution at a quasi-equilibrium after 5,000 generations. Longer simulation runs do not qualitatively change the phenotype distribution for a given parameter combination. (a) With no disruptive selection and no assortative mating ($\gamma = 0$, $r = 0$), phenotypic variance follows a binomial (approximately normal) distribution whose variance depends on the level of polymorphism at the 40 additive loci, maintained by mutation-drift balance. (b) Using a combination of parameters close to the strongest disruptive selection and assortative mating observed in stickleback ($2\gamma = 0.15$, $r = 0.5$), the population attains modestly higher phenotypic variance than in (a) but remains unimodal. (c) Larger parameter values ($2\gamma = 0.3$, $r = 0.6$) rapidly lead to a weakly bimodal phenotype distribution that does not resolve itself into separate species even after 50,000 generations. (d) Speciation can occur when disruptive selection and assortment are near the upper end of plausible empirical values ($2\gamma = 0.4$, $r = 0.8$).
speciation is possible. However, for the majority of stickleback populations such reinforced assortative mating would still not facilitate speciation. An important subject for future empirical investigation is whether disruptive selection really does drive the evolution of increased assortative mating.

6. It Is Not That Easy: Additional Constraints on Speciation in Stickleback

If reinforcement could push the most disruptive populations into speciation-prone parameter space, one might ask why this has not actually happened. The answer is that there may be additional constraints on sympatric speciation, many of which are not typically considered in speciation models. For example, increased assortment may be inhibited if selection is variable through time, or if the population evolves an alternative solution to disruptive selection. In the last section of this paper, I summarize evidence for both kinds of speciation-inhibiting factors in stickleback.

6.1. Stability of the Fitness Landscape. Theoretical models of speciation typically assume populations are subject to persistent disruptive selection despite general evidence for temporal fluctuations in fitness landscapes [100, 101] (though some fluctuations can be due to sampling noise). Of the 14 lakes studied by Bolnick and Lau [24], First and McNair Lakes were among the ones with the strongest disruptive selection (Figure 2). A second sample of these two lakes, two years later, found significant stabilizing selection in First Lake and directional selection in McNair [22]. The reason for this interannual variation is not clear. One hypothesis is that fluctuating population density [102] (or resource availability) alters the strength of competition and thus the fitness landscape [48]. For instance, a long-term study of a population of Eurasian Perch (Perca fluviatilis) found shifts in the fitness landscape associated with two-order-of-magnitude population density cycles. At high density, perch experienced disruptive selection, which changed to stabilizing or directional selection during periods of low density [103]. Changes in selection gradients might also reflect interannual variation in predation regimes [50, 104–107] or the strength of interspecific competition [65]. Such variable selection regimes would undermine sympatric speciation [108, 109] and might instead favor the evolution of phenotypic plasticity [110]. In fact, temporal changes in the fitness landscape may explain the introgressive collapse of formerly distinct species pairs of stickleback [83].

6.2. Alternative Forms of Diversification. Frequency-dependent competition gives rise to disruptive selection, because the resource distribution can create a broader phenotypic variance than presently found in the population [111–113]. Evolution will thus favor any of a number of evolutionary changes that increase the population’s phenotypic variance; assortative mating is just one of several mechanisms that can increase this variance [114]. If some other form of diversification evolves first and mitigates the disruptive selection, sympatric speciation will be less likely [115].

The most straightforward outcome of disruptive selection is increased genetic variance within a phenotypically unimodal population (Figure 5(b)). Consistent with this outcome, derived lacustrine populations of stickleback exhibit higher phenotypic variance (particularly for gill raker traits under disruptive selection) compared to marine stickleback that represent a putative ancestral character state [116]. It is important to note, however, that these results reflect phenotypic variances, and it is not known to what extent among-population differences in phenotypic variances are matched by underlying differences in genetic variances.

Disruptive selection due to intraspecific competition can also drive the evolution of ecological sexual dimorphism [115, 117, 118], which can inflate trait variance. This intraspecific ecological character displacement occurs under the same theoretical conditions as sympatric speciation [115]. However, dimorphism can evolve more quickly than assortative mating, reducing disruptive selection to the point where sympatric speciation is inhibited [115] though this antagonism can be relaxed if disruptive selection acts along multiple trait axes [119]. Stickleback exhibit sexual dimorphism with respect to diet and associated trophic morphology such as gill raker length [24, 104, 120, 121]. This dimorphism is maximized in intermediate sized lakes where disruptive selection is typically strongest [24], supporting the notion that disruptive selection leads to dimorphism [115, 119]. However, controlling for lake size, there is a negative relationship between present-day disruptive selection and dimorphism [24], implying that once dimorphism evolves the fitness landscape becomes flatter, which may inhibit speciation [115]. These results seem to confirm the notion that sexual dimorphism can evolve quickly and mitigate disruptive selection, thereby potentially reducing the capacity for sympatric speciation.

Another possibility is that disruptive selection favors the evolution of phenotypic plasticity [122], allowing individuals to shift onto underused resources within a single generation rather than over evolutionary time [110]. Optimal foraging theory (OFT) is predicated on the idea that individuals are able to flexibly choose prey to maximize their expected fitness [123], accepting lower-value resources as preferred prey become scarce [124]. Applied to phenotypically variable populations, OFT predicts that strong competition might lead to behavioral rather than evolutionary diversification [125]. Consistent with this prediction, experimentally elevated stickleback densities led to greater population niche width and greater among-individual diet variation over the space of a mere two weeks [41, 56]. Thus, behavioral plasticity clearly does provide a mechanism for diversification in response to changing fitness landscapes. Morphological plasticity may play a similar role albeit over a slower time scale as plasticity relies on somatic growth and reshaping. Stickleback do exhibit diet-induced plasticity, which is greatest in solitary populations (Svanbäck and Schluter, unpublished).

To summarize, stickleback concurrently exhibit several forms of within-population diversification: increased phenotypic variance, dimorphism, behavioral plasticity, and
morphological plasticity. Any or all of these may increase trophic diversity in ways that mitigate disruptive selection and can inhibit speciation.

7. Conclusions

The past two decades have seen a burst of renewed research on sympatric speciation. Collectively, this body of literature suggests that sympatric speciation (1) probably has occurred in at least a few cases [3, 5, 6] but is still relatively rare [97, 126] and difficult to prove and (2) is “theoretically plausible” [127] but requires very specific conditions [25]. An important next step is to fuse the theoretical and empirical work (e.g., [12, 13]), by empirically evaluating the biological plausibility of the key theoretical assumptions in particular model organisms [97]. This shifts empirical focus away from putative cases of sympatric speciation to a more general program of jointly measuring key parameters (e.g., selection strength, spatial structure and gene flow, strength of assortative mating, and identifying phenotypic targets of mate choice). These estimates can then be used to build a more biologically informed body of theory (e.g., [12, 13]) or to identify those populations best suited to the various forms of sympatric diversification.

In stickleback, the good news for theoreticians is that the joint occurrence of (weak) disruptive selection and assortative mating validates long-standing assumptions. Meta-analyses suggest that both phenomena are quite common [31, 33], though weak and perhaps temporally variable [100, 101]. While the joint action of disruptive selection and assortment is less well established, the work reviewed above indicates that the processes do co-occur in lake populations of stickleback. The bad news for theory is that both forces are typically too weak to drive speciation. As is often the case in ecological and evolutionary theory, model predictions are sensitive to the precise parameter values used. Consequently, rigorous tests of theory requires models framed using measurable parameters coupled with suitable empirical data. Theoretical models of speciation also typically ignore temporal fluctuations in selection, and alternative forms of diversification, such as we see in stickleback. Such constraints do not make the theory wrong so much as incomplete, echoing Gavrilets’ statement that “it is not that easy” [25].

The absence of sympatric speciation in stickleback is not necessarily an indictment of sympatric speciation theory. Indeed, it is the comparison of theory with empirical parameter values that helps illuminate why speciation may not readily occur. Furthermore, existing models have made a variety of related testable predictions, such as the links between competition, habitat diversity, and sexual dimorphism, which have been empirically tested [24, 48, 56, 65, 119]. Thus, models of sympatric speciation may be an illustration of G. E. P. Box’s statement that, “all models are wrong, but some are useful” [128]. Perhaps continued controversy over sympatric speciation will prove useful as well, regardless of its prevalence in nature, particularly if the debate spurs more extensive empirical measurement of generally important evolutionary forces.

Appendix

Simulation of Phenotypic Evolution under Disruptive Selection and Assortative Mating

I used stochastic simulations of an individual-based model to explore the potential for sympatric speciation across a range of magnitudes of disruptive selection and assortative mating. Sympatric speciation has been widely explored with both stochastic and numerical simulations and analytical theory (summarized in [97]), but these models tend not to be framed in terms of empirically measurable parameters. I, therefore, focus here on describing a model that can be framed in terms of disruptive selection gradients and male-female trait correlations.

I consider a population of N haploid individuals characterized by a phenotype subject to both disruptive selection and assortative mating. Each individual’s phenotype is a quantitative trait that depends on the additive effect of 40 independently assorting loci of small and equal effect. Initially, each locus is assumed to be polymorphic with equal frequencies of two alleles of effect size 0 or 1. Thus, the phenotype space ranges from a minimum of 0 to a maximum of 40 with an initial mean of 20 and variance 10. Individuals were created by randomly drawing a set of 40 allele values.

The general results described here are qualitatively robust to using smaller numbers of loci [63]. The traits under disruptive selection in stickleback are known to be polygenic, with multiple QTL none of which have substantial effect sizes [63]. Consequently, using a moderate to large number of loci is probably appropriate.

In most sympatric speciation models, disruptive selection is an emergent outcome of some assumed ecological process such as intraspecific competition [129]. This adds a degree of realism, to the extent that the ecological process is biologically justified, but makes it difficult to directly control the strength of disruptive selection as measured empirically. Here, I ignored the ecological mechanism underlying selection and instead used a specified disruptive selection gradient to calculate the corresponding fitness for each phenotype in the population. Specifically, I first standardized the population’s phenotype distribution to a standard normal (mean = 0, standard deviation = 1). Next, individuals’ fitness is calculated as

$$w(x) = w_{\text{center}} + \beta x + \gamma x^2,$$

(A.1)

where $x$ is an individual’s standardized phenotype, $w_{\text{center}}$ is the fitness of the average phenotype, $\beta$ is the linear selection gradient (assumed to be 0), and $\gamma$ is the quadratic selection gradient. Fitness was then standardized by dividing every individuals’ fitness $w(x)$ by the maximal fitness, yielding a value that falls within the range 0 to 1. This fitness is used as the probability the individual survives viability selection.

This selection scheme ensures that the minimum of disruptive selection tracks the phenotype mean, preventing the population from evolving away from the fitness minimum. The stabilized fitness minimum roughly mimics the behavior of models of frequency-dependent competition that generate stable fitness minima required for speciation [15]. Finally,
a uniform random number was drawn for each individual; if the random number was less an individual’s \( w(x) \), the individual was retained for mating.

Following a round of selection, the surviving individuals select mates and reproduce. Individuals are randomly assigned a sex assuming an equal sex ratio. One female and one male are randomly drawn from the pool of available survivors, and we calculated the probability the individuals mate based on their phenotypic similarity (see next paragraph for details). If a random uniform number fell below this probability, they produced one offspring and were returned to the pool of breeders. If not, they were simply returned to the breeders, and a new pair was randomly selected. Whether or not a pair mated, they were, therefore, able to subsequently remate. This scheme allows for some sexual selection to occur; for instance, with strong positive assortative mating, rare phenotypes may be unable to find acceptable mates. This mate pairing was iterated until the desired population size of offspring was reached (allowing a constant population size throughout the simulations), at which point all adults died and a new generation began.

The typical speciation model calculates a probability the female accepts the male, given their phenotypic difference

\[
P(mating) = \exp \left( - \frac{(x_{\text{male}} - x_{\text{female}})^2}{2\sigma^2} \right), \tag{A.2}
\]

where \( x \) is the phenotypic value of the male and female being evaluated and \( \sigma^2 \) is the variance of the mate probability function. This variance measures how quickly mating probability drops off with phenotypic difference. Most speciation models either used a set value of \( \sigma^2 \) or allow \( \sigma^2 \) to evolve as a female phenotype (choosiness; e.g., [9]). However, this parameter is difficult to measure empirically in the field. Instead, I ran simulations with a specified male-female correlation, \( r \), and used a known relationship to convert \( r \) to \( \sigma^2 \) which we subsequently used in (A.2) to calculate the mating probability for a given male-female pair. Assuming a Lande-style relative mate choice function [130], the variance of the mate choice function and the phenotypic standard deviation determine the resulting correlation between mated pairs

\[
r = \frac{\sigma_m \sigma_f}{\sqrt{\sigma_m^2 + \sigma_f^2}}, \tag{A.3}
\]

See [131]. This can be rearranged to calculate a value for \( \sigma^2 \) given the phenotypic variance for a trait found in both sexes (\( \sigma_x = \sigma_m = \sigma_f \)) and a desired \( r \)

\[
\sigma_a = \left[ \frac{\sigma^4}{r^2} - \sigma_x^4 \right]^{1/2}. \tag{A.4}
\]

When individuals did mate, I generated one offspring whose genotype is a random sample of alleles from the two parents. That is, for each locus the offspring receives an allele randomly from one parent. To maintain genetic variation, I allowed a mutation process that inverts the phenotypic value of the inherited allele (0 to 1, or 1 to 0) with a specified probability (here, 0.0001, which was chosen because it maintains fairly constant phenotypic variance in the face of drift in simulations without selection or assortment).

I iterated this life cycle (selection, then rounds of mating until the population size was met) for a specified number of generations. At each time step, I recorded the phenotype distribution. At the end of the simulation, speciation was said to have occurred if the phenotype distribution was bimodal, and intermediate phenotypes had a frequency less than 5% that of the lower of the two modes. I iterated through a range of parameter space (\( y \) from 0 to 0.5 in increments of 0.5, and \( r \) from 0 to 0.95 in increments of 0.5). For each parameter combination, I replicated a 1000 generation simulation 10 times and recorded whether or not speciation occurred. I also recorded the phenotypic variance of the ending trait distribution. Simulations were conducted in R [132], and the code is available from the author on request.

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References


Research Article

Ecological Divergence and the Origins of Intrinsic Postmating Isolation with Gene Flow

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The evolution of intrinsic postmating isolation has received much attention, both historically and in recent studies of speciation genes. Intrinsic isolation often stems from between-locus genetic incompatibilities, where alleles that function well within species are incompatible with one another when brought together in the genome of a hybrid. It can be difficult for such incompatibilities to originate when populations diverge with gene flow, because deleterious genotypic combinations will be created and then purged by selection. However, it has been argued that if genes underlying incompatibilities are themselves subject to divergent selection, then they might overcome gene flow to diverge between populations, resulting in the origin of incompatibilities. Nonetheless, there has been little explicit mathematical exploration of such scenarios for the origin of intrinsic incompatibilities during ecological speciation with gene flow. Here we explore theoretical models for the origin of intrinsic isolation where genes subject to divergent natural selection also affect intrinsic isolation, either directly or via linkage disequilibrium with other loci. Such genes indeed overcome gene flow, diverge between populations, and thus result in the evolution of intrinsic isolation. We also examine barriers to neutral gene flow. Surprisingly, we find that intrinsic isolation sometimes weakens this barrier, by impeding differentiation via ecologically based divergent selection.

1. Introduction

Speciation is a central topic in evolutionary biology and occurs as inherent barriers to gene flow evolve between formerly interbreeding populations. As data accumulate, it is becoming more apparent that many different forms of barriers to gene exchange (reproductive isolation) can be involved in speciation [1–4]. One barrier that has received much attention, both historically and in recent work on the genes causing reproductive isolation, is low hybrid fitness due to genetic incompatibilities (intrinsic postmating isolation) [5–9]. The evolution of intrinsic reproductive isolation has received so much attention because it has been one of the key mysteries of speciation. How is it possible, if hybrids are unfit, to evolve from the high-fitness genotype of one species to the high-fitness genotype of the other species without passing through a low-fitness intermediate?

The most famous answer to this question is attributed to the insights of Bateson, Dobzhansky, and Muller [10–13]. Specifically, intrinsic isolation often arises from between-locus genetic incompatibilities: alleles that function well within species are incompatible with one another when brought together for the first time in the genome of a hybrid (see [14] for review). Genetic architectures of this form are known as Dobzhansky-Muller (DM) incompatibilities (illustrated in Table 1) and, in modern parlance, allow populations to evolve reproductive isolation without passage through a fitness valley that would be opposed by selection. The accumulation and origin of genetic incompatibilities, as well as their effects on levels of gene flow upon secondary contact, have received much attention (Table 2 for review). Along these lines, it can be difficult for intrinsic postzygotic barriers to evolve in the face of gene flow (i.e., in parapatry or sympatry). The logic is that in interbreeding populations, deleterious genotypic combinations will
Table 1: Simple Dobzhansky-Muller incompatibility. With this type of genetic architecture, it is possible to evolve from one high fitness genotype (AB) to another (ab) without passing through any fitness valleys (by AB → aB → ab). Matings between two high fitness types yield unfit hybrids (i.e., AB × ab produces the unfit type Ab).

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be created and then quickly eliminated by selection [3]. However, divergent selection in general might overcome gene flow to cause population divergence [15–20]. Thus, one might imagine scenarios for the origin of intrinsic incompatibilities in the face of gene flow where divergent selection drives the evolution of incompatibilities, perhaps in the face of gene flow. Such scenarios represent the process of “ecological speciation”, where reproductive isolation evolves as populations adapt to different ecological environments, as a simple and incidental by-product of adaptive genetic divergence [21–26].

Ecological speciation is expected to result in reduced fitness of immigrants into foreign environments and hybrids (i.e., extrinsic isolation), due to an ecological mismatch between the phenotype of immigrants and hybrids and local environmental conditions [4, 42]. However, the evolution of any type of reproductive barrier might evolve as a by-product of divergent adaptation. Thus, the negative gene-by-gene interactions that cause intrinsic postmating isolation might also arise via adaptive genetic divergence if, for example, the different alleles favored in divergent habitats are incompatible with one another. Along these lines, intrinsic isolation may evolve in the face of gene flow, as long as the diversifying effects of divergent selection can overcome the homogenizing effects of gene flow. Verbal arguments along these lines were made quite some time ago, for example, in the writings of Sir Ronald Fisher [43] and in more recent reviews [44].

Indeed, there is empirical evidence that ecological divergence can drive the evolution of intrinsic postmating isolation. Consider the classic case of ecological adaptation and intrinsic hybrid sterility in *Mimulus* monkey-flowers adapted to different soil types [45]. In this example, crossing experiments revealed that a yet unidentified gene conferring tolerance to copper in the soil (or a gene tightly physically linked to it) interacts with a small number of genes in another population to generate intrinsic hybrid incompatibility. Thus, genes conferring adaptation to copper contribute to postmating isolation. Evidence for a role for divergent adaptation in the evolution of intrinsic reproductive isolation also stems from broad comparative studies in fish [46] and insects (fruit flies and moths/butterflies [25]). These studies considered multiple species pairs and examined the relationship between indices of the degree of ecological divergence between species pairs and the extent of intrinsic postmating isolation between species pairs. Both studies report positive correlations between ecological divergence and intrinsic isolation, independent from time (i.e., after controlling for time since species divergence using genetic distance between species pairs) and report positive correlations between ecological divergence and intrinsic isolation. Finally, experimental and genetic studies in yeast also implicate divergent adaptation in promoting the evolution of intrinsic incompatibilities [47, 48].

Although these arguments and observations about how divergent selection might overcome gene flow to generate intrinsic incompatibilities have been around for some time and make intuitive sense, there has been little formal theoretical exploration of the specific conditions under which genetic incompatibilities might originate during ecological speciation with gene flow. As noted by Coyne and Orr [3], “we currently lack any theory telling us how much intrinsic isolation can evolve under a given level of hybridization” (page 177). Here we aim to help fill this theoretical gap by exploring the evolution of intrinsic isolation in the face of gene flow using a combination of analytical and simulation models. We examine how migration, selection, and recombination interact to affect this process. We also examine how the evolution of intrinsic isolation affects rates of neutral gene flow. The results help clarify that ecological adaptation can generate intrinsic incompatibilities.

Our treatment differs from and extends important past models of DM incompatibilities in several ways. For example, classic theoretical treatments of intrinsic incompatibilities (e.g., [27, 28, 33]) generally did not consider the evolutionary processes driving genetic divergence in the underlying genes involved; allopatry is explicitly assumed or implied, and incompatibilities evolve by drift or some unspecified form of selection. Newer models include selection, but it is usually not divergent between environments (see Table 2 for consideration of past models). Our focus on the origin of incompatibilities via divergent selection and its interaction with gene flow sets our treatment apart from most previous models. While many past studies involving selection were completely simulation based, our model helps to answer the call for analytical treatments of speciation models [49].

Our models are extensions of classical theoretical work on geographic clines by Clarke [16] and Endler [18] and of more recent models by Gavrilets [14], all of which did consider divergent selection in some context. Indeed, the hybrid zone literature in general discussed how geographic clines generated by divergent selection could be steepened by the spread of modifier loci that also contribute to DM incompatibilities [19, 20]. Our work is most closely related to that of Clarke [16], though our goals are quite different. He was interested in understanding how clines of key morphological characters (controlled by a single locus) could be sharpened by epistatic modifiers. To our knowledge, he was the first to explicitly model how epistatic modifiers can build upon differences established by divergent selection, though he did not explicitly discuss reproductive isolation or measure barriers to gene flow in other parts of the genome.

Our work thus extends previous models by considering (1) the joint evolution of extrinsic versus intrinsic isolation during ecological divergence, (2) the explicit effects of divergent ecological selection on epistatically interacting loci,
Table 2: In chronological order, a summary of some major previous models of Dobzhansky-Muller Incompatibilities (DMIs). Uniform selection refers to scenarios where selection occurs (i.e., some alleles or genotypic combinations are more fit than others), but selection is not divergent between different ecological environments (i.e., no environmental variation). Uniform selection thus fits the “mutation-order speciation” model recently advanced by Schluter [26]. In contrast, divergent selection fits the ecological speciation model. IBD: Isolation-by-distance.

<table>
<thead>
<tr>
<th>Model</th>
<th>Process driving divergence</th>
<th>Migration and gene flow</th>
<th>Discrete patches (populations)</th>
<th>Method</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>[16]</td>
<td>Divergent selection</td>
<td>Yes</td>
<td>Clinal divergence rather than discrete patches</td>
<td>Analytical</td>
<td>Divergent selection is on major locus, modifier locus affects the fitness of alleles at the major locus, but is not under divergent selection itself; barrier to neutral gene flow not examined. Also, focus of the model is how the modifier, once fixed, alters the shape of the cline, rather than on how the modifier fixes in the first place.</td>
</tr>
<tr>
<td>[18]</td>
<td>Divergent selection</td>
<td>Yes</td>
<td>Clinal divergence rather than discrete patches</td>
<td>Analytical</td>
<td>Divergent selection is on major locus, modifier locus causes DMI but is not under divergent selection itself; barrier to neutral gene flow not examined.</td>
</tr>
<tr>
<td>[27]</td>
<td>Stochastic (drift)</td>
<td>No</td>
<td>Yes</td>
<td>Simulation</td>
<td>Drift fixed new mutations in small populations; two loci considered.</td>
</tr>
<tr>
<td>[28]</td>
<td>Unspecified</td>
<td>No</td>
<td>Yes</td>
<td>Analytical</td>
<td>The classic paper on the accumulation of DMIs. No assumptions are made about evolutionary causes of substitutions driving DMIs.</td>
</tr>
<tr>
<td>[29, 30]</td>
<td>Uniform selection, not explicitly divergent</td>
<td>Yes</td>
<td>Yes</td>
<td>Analytical</td>
<td>Migration can allow the spread of a gene combination whose component alleles are not individually favored, but the conditions for this are restricted (low migration, strong advantage for particular genotypic combinations).</td>
</tr>
<tr>
<td>[31]</td>
<td>Uniform selection or drift</td>
<td>No</td>
<td>Yes</td>
<td>Analytical and simulation</td>
<td>Population subdivision has no effect on time to speciation by drift. Under selection, speciation occurs more rapidly between two large populations than between many small ones.</td>
</tr>
<tr>
<td>[32]</td>
<td>Unspecified; populations initially fixed for different incompatible alleles</td>
<td>Yes</td>
<td>Yes, stepping stone</td>
<td>Analytical</td>
<td>Focused on the effect of intrinsic incompatibilities on the barrier to neutral gene flow.</td>
</tr>
<tr>
<td>[33]</td>
<td>Unspecified</td>
<td>No</td>
<td>Yes</td>
<td>Analytical</td>
<td>Adds stochasticity of molecular evolution to the results of Orr [28].</td>
</tr>
<tr>
<td>[34]</td>
<td>Uniform selection</td>
<td>Yes</td>
<td>Yes, between neighboring patches</td>
<td>Simulation</td>
<td>Speciation in subdivided populations occurs most rapidly when there is some migration (which spreads incompatible alleles); many (250) loci are considered.</td>
</tr>
<tr>
<td>[35]</td>
<td>Uniform selection</td>
<td>Yes</td>
<td>Yes</td>
<td>Simulation</td>
<td>Strong selection counters the inhibitory effects of gene flow, permitting the evolution of intrinsic postmating isolation.</td>
</tr>
<tr>
<td>[36]</td>
<td>Uniform selection</td>
<td>Yes</td>
<td>Yes</td>
<td>Analytical</td>
<td>Migration occurs through a single, spatially structured population and affects the accumulation of DMIs.</td>
</tr>
<tr>
<td>[37]</td>
<td>Uniform selection</td>
<td>Yes</td>
<td>Yes</td>
<td>Analytical</td>
<td>Loci causing DMIs themselves are under selection (i.e., new alleles at these loci can be favored), but selection is uniform. Barrier to gene flow is considered, including the effect of chromosomal inversions.</td>
</tr>
<tr>
<td>(Gavrilets [14]) and previous models by same author (e.g., [38, 39])</td>
<td>Divergent selection, uniform selection, or drift</td>
<td>Yes</td>
<td>Yes</td>
<td>Analytical</td>
<td>Incompatible alleles advantageous in one environment but neutral in other (thus wider range of scenarios examined in current study), barrier to gene flow examined.</td>
</tr>
<tr>
<td>[40]</td>
<td>Uniform selection</td>
<td>Yes</td>
<td>No, clinal with IBD</td>
<td>Simulation</td>
<td>IBD and outbreeding depression were sufficient to drive speciation, in the absence of ecological differences.</td>
</tr>
</tbody>
</table>
(3) the examination of the barrier to neutral gene flow caused by intrinsic isolation, and (4) a focus on the initiation of divergence.

Our model is similar to that of Gavrilets [32] in its examination of barriers and intrinsic isolation (point (3) above). However, Gavrilets [32] assumes divergence in allopatry (via some unspecified process) and begins his analysis after intrinsic isolation already exists. In contrast, our model focuses on how divergent ecological selection causes intrinsic isolation to originate: ecological divergence in our models establishes the initial genetic divergence upon which incompatibilities can be built. As discussed below, the models yield some novel and counterintuitive predictions. For example, we find that the evolution of intrinsic isolation need not necessarily strengthen the barrier to neutral gene flow; whether this occurs is dependent on the effect of intrinsic isolation on the genetic differentiation causing extrinsic isolation.

2. Model

If the level of migration is low compared to the strength of divergent selection, populations inhabiting different environments can quickly become differentiated with respect to genes underlying key ecological traits [3, 50, 51]. Intrinsic isolation can then arise between taxa because (1) the genes underlying key ecological traits also generate incompatibilities themselves, or (2) subsequent mutations that are not necessarily integral to ecology are incompatible with the altered genetic backgrounds generated by the initial adaptive changes. Attempting to model the evolution of reproductive isolation in a spatially heterogeneous environment with multilocus genotypes can quickly lead to an overly complex model with numerous parameters. To minimize these difficulties, we initially focus on the simplest possible model that allows us to illustrate some key points: a two-locus haploid model with two populations connected by migration. We then show through simulation that the major results apply in diploid systems.

We are interested in exploring the following scenario. Consider a species in which the AB haplotype is both fit and common in Population 1 (Figure 1). A second population of this species (Population 2) becomes established in a new habitat in which there is ecological selection favoring an a allele. Assuming migration levels are low relative to the strength of divergent selection, aB becomes the common genotype in Population 2. Imagine that the B locus is an epistatic modifier of the A locus. While the B allele works well with the A allele, the b allele improves the fitness of individuals carrying an a allele. For example, the a allele may be a gene that confers tolerance to high levels of copper, as in the monkey-flower example, but also incurs some pleiotropic cost. The b allele might mitigate this cost, thus improving the intrinsic fitness of individuals carrying an a allele. The b allele, however, could interact negatively with the A allele, regardless of the environment, uncovering deleterious consequences of the A allele normally masked by the B allele. If AB is common in Population 1 and aB is common in Population 2, then recombinants between them will be less fit on average, even in the absence of copper. This would be considered intrinsic isolation.

Specifically, we assume that the A locus controls a key ecological trait and is subject to divergent ecological selection. In Population 1, ecological selection reduces the fitness of individuals with an a allele by an amount s1; in Population 2, ecological selection reduces the fitness of individuals with an A allele by an amount s2. The B locus is an epistatic modifier of the A locus. Specifically, the b allele improves the intrinsic fitness of the a genotype by an amount εa, perhaps by mitigating some ecologically independent pleiotropic cost associated with a. The b allele interacts negatively with the A allele, reducing intrinsic fitness by εb. There may also be direct ecological selection on the B locus. If so, b genotypes have a fitness disadvantage of t1 in Population 1 whereas B genotypes have a disadvantage of t2 in Population 2. These fitness effects are shown explicitly in Table 3.

### Table 2: Continued.

<table>
<thead>
<tr>
<th>Model</th>
<th>Process driving divergence</th>
<th>Migration and gene flow</th>
<th>Discrete patches (populations)</th>
<th>Method</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study</td>
<td>Divergent selection</td>
<td>Yes</td>
<td>Yes</td>
<td>Analytical and simulation</td>
<td>Focused on the origin of DMI due to different, incompatible mutations diverging between populations adapting to identical selection regimes. Gene flow strongly impeded strong divergence via such a &quot;mutation-order&quot; process (c.f., Schluter [26])</td>
</tr>
</tbody>
</table>

### Table 3: Fitnesses of the four haplotypes in the full model. For Case 1, t1 = t2 = 0.

<table>
<thead>
<tr>
<th>Population 1</th>
<th>A</th>
<th>a</th>
<th>A</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>1</td>
<td>1 - s1</td>
<td>(1 - s2)(1 - t2)</td>
<td>1 - t2</td>
</tr>
<tr>
<td>( b(1 - t1)(1 - \epsilon_a) (1 - t1)(1 - s2)(1 + \epsilon_a) (1 - s2)(1 - \epsilon_a) 1 + \epsilon_a )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Divergent ecological selection causes divergence between populations with respect to the A locus. The modifier b evolves in Population 2 because it is beneficial in combination with a. Direct ecological selection may also favour the b allele.

Figure 1: Divergent selection and the evolution of intrinsic isolation. Time 0: Two populations, connected by migration inhabit different environments. Both are initially fixed for the AB genotype. Time 1: Divergent ecological selection has caused divergence at the A locus, with the a allele evolving to high frequency in Population 2. Time 2: The modifier b evolves in Population 2, either because it ameliorates a pleiotropic cost of the a allele (Model 1) or because the B locus also experiences divergent ecological selection (Model 2).

We assume that migration occurs following selection within each population. The migration rate of individuals into Population 1 is \( m_1 \), and the rate into Population 2 is \( m_2 \). Technically, this is accomplished as follows. Both populations contribute to the migrant pool, but they may do so differently. A fraction \( c \) of individuals in the migrant pool comes from Population 1, while the remainder \((1 - c)\) comes from Population 2. In each population, a fraction \( m \) of all individuals come from the migrant pool with the remainder being residents. This means \( m_1 = m(1 - c) \) and \( m_2 = cm \). A number of ecological scenarios can be described by the appropriate choice of \( m \) and \( c \). For example, if Population 1 is much larger than Population 2 such that migration is primarily from Population 1 into Population 2, then \( c \) would be close to 1. Throughout, we have assumed soft selection (i.e., a deme's contribution to the migrant pool is independent of its genetic composition/fitness).

Following migration, mating occurs randomly within each population. The rate of recombination between the two loci is \( r \). The populations are assumed to be sufficiently large that drift can be ignored. Recursion equations describing this life cycle (selection, migration, reproduction) were created in the standard way and are available as supplementary material in the form of a Mathematica notebook (see Supplementary Material available online at doi: doi:10.1155/2011/435357).

A number of assumptions were made in order to make analytical progress. We assume that both ecological and intrinsic selections are weak in an absolute sense but strong relative to migration. Specifically, the selection parameters \( s_1, s_2, t_1, t_2, \epsilon_A, \) and \( \epsilon_a \) are \( O(\xi) \), and \( m \) is \( O(\xi^2) \) where \( \xi \ll 1 \). We performed the analysis with both tight and loose linkage (i.e., assuming \( r \) is \( O(\xi) \) or \( O(\xi^0) \)). The results represented assume tight linkage unless otherwise stated. Differences between the analyses are noted when they are important (see supplementary material for details). We also conducted numerical simulations for comparison with our analytical results. These simulations indicate that the analytical results are reasonably robust to these assumptions provided that migration does not become too strong relative to selection (see Results).
3. Results

Our primary results are most easily seen in a simplified case in which there is no ecological selection on the epistatic modifier. We present this case below. The full model including both ecological selection on the modifier and the complete range of epistatic effects is discussed in the supplementary material and presented in the numerical results below.

Case 1 (Epistatic Trade-off). In this case, the epistatic modifier allele $b$ improves the fitness of individuals carrying the $a$ allele by an amount $\epsilon_a$, but reduces the fitness of $A$ individuals by an amount $\epsilon_A$. There is no divergent ecological selection acting on the $B$ locus, $t_1 = t_2 = 0$.

3.1. Equilibrium Allele Frequencies and Differentiation. Divergent ecological selection causes the populations to differentiate with respect to the ecologically important locus $A$. Assuming the $b$ allele is initially absent, the equilibrium frequencies for $A$ at migration-selection balance in Populations 1 and 2, respectively, are given by

$$p_{A,1} = 1 - \frac{m_1}{s_1} + O(\xi^2),$$

$$p_{A,2} = \frac{m_2}{s_2} + O(\xi^2).$$

We can quantify the differentiation between the two populations at this locus as

$$\delta_{A;b \ fixed} = p_{A,1} - p_{A,2} = 1 - \frac{m_1}{s_1} - \frac{m_2}{s_2} + O(\xi^2).$$

The $b$ allele can invade the system by first spreading in Population 2, where $b$ is favored if the $a$ allele is sufficiently common there. This will be the case provided that migration into this population is not too high,

$$m_2 < \frac{s_1(r + s_1 + \epsilon_A + \epsilon_a)e_a}{r(s_2 + \epsilon_A + \epsilon_a)}.$$  \hspace{1cm} (3)

Following invasion, the equilibrium frequencies of the $B$ allele in the two populations are

$$p_{B,1} = 1 - \frac{m_1(r + \epsilon_A)}{\epsilon_A(r + s_1 - \epsilon_a)} + O(\xi^2),$$

$$p_{B,2} = \frac{m_2(r + \epsilon_A + \epsilon_a)}{\epsilon_a(r + s_2 + \epsilon_a)} + O(\xi^2).$$  \hspace{1cm} (4)

At this equilibrium, $b$ is common in Population 2. Because $b$ enhances the fitness advantage of $a$ over $A$, the equilibrium frequencies of $A$ change due to the invasion of $b$. The leading order approximations for the equilibrium frequencies of $A$ at this new equilibrium are

$$p_{A,1} = 1 - \frac{m_1(r + s_1)}{s_1(r + s_1 - \epsilon_a)} + O(\xi^2),$$

$$p_{A,2} = \frac{m_2(r + s_2 + \epsilon_A + \epsilon_a)}{(s_2 + \epsilon_A + \epsilon_a)(r + s_2 + \epsilon_a)} + O(\xi^2).$$

The invasion of $b$ alters the equilibrium frequency of $A$ in Population 2, thereby changing the degree of differentiation between the two populations at the ecologically important locus. The change in differentiation is quantified by

$$\Delta \delta = \delta_{A;b \ present} - \delta_{A;b \ fixed}$$

$$= -m_1 \frac{\epsilon_a}{s_1(r + s_1 - \epsilon_a)} - m_2 \frac{r + s_2 + \epsilon_A + \epsilon_a}{(s_2 + \epsilon_A + \epsilon_a)(r + s_2 - \epsilon_a)} - \frac{1}{s_2} + O(\xi^2).$$  \hspace{1cm} (6)

When migration is weak relative to selection, there is strong divergence at the $A$ locus and the evolution of the $b$ locus has very little effect on the magnitude of divergence (i.e., $|\Delta \delta| \ll \delta$). The expression above tends to be negative, implying that the evolution of an epistatic modifier reduces differentiation at the $A$ locus. This provides our first hint that the evolution of incompatibilities can reduce barriers to gene flow (see below).

The equilibrium described above is stable under the assumption that migration is weak relative to selection. When linkage is loose ($r \gg s$), analysis of the largest eigenvalues of the Jacobian matrix indicates that stability requires the following conditions

$$m_1 < \min \left[ \frac{s_1 \epsilon_A}{2 \epsilon_A + \epsilon_a}, \frac{s_1 \epsilon_A}{\epsilon_A + \epsilon_a + s_1} \right],$$  \hspace{1cm} (7)

$$m_2 < \frac{(\epsilon_a + \epsilon_A + s_2) \epsilon_a}{2(\epsilon_a + \epsilon_A) + s_2}. $$

The reasons for instability are fairly intuitive. With high migration, the $b$ allele will often find itself in the wrong population and with the wrong (i.e., $A$) allele. Its fate is determined by its epistatic interactions. When $\epsilon_A$ is low, $b$ fixes in both populations because the disadvantage of $Ab$ relative to $AB$ in Population 1 is small compared to the advantage of $ab$ relative to $AB$ in Population 2. If $\epsilon_a$ is high, $ab$ can fix in both populations because $ab$ is more fit than $AB$ across the metapopulation (the average fitness over both environments is the relevant fitness measure if populations are well mixed). If $\epsilon_a$ is low and $\epsilon_A$ is high, then $B$ fixes in both populations because the disadvantage of $Ab$ relative to $AB$ in Population 1 is large compared to disadvantage of $ab$ relative to $ab$ in Population 2. While the stability conditions above serve as a useful heuristic, they should be regarded with caution as they are obtained from analyses assuming that migration is weak relative to selection (i.e., instability occurs outside the region where the model assumptions are valid).

The stability conditions outlined above assume loose linkage. Stability is also influenced by the recombination rate $r$ when linkage is tight (see Mathematica file provided as online supplementary material). In the simulations described below, we see that tight linkage tends to destabilize the two-locus polymorphism. In the remaining sections we focus on parameter regions where the polymorphic equilibrium is stable.

3.2. Intrinsic Isolation. Intrinsic isolation is usually measured empirically by comparing the fitness of hybrids to the average
fitness of parental types. Typically, such experiments are done in the lab where ecological selection is typically absent, or at least different than in either of the environments from which the parents are collected. Even in the lab, the ecological trait controlled by the A locus may affect fitness. To account for this, we model extrinsic selection for or against the a allele in the lab by the variable $s_{ab}$, where the value of $s_{ab}$ may be negative, positive, or zero. Assuming that the intrinsic fitness effects represented by $e_A$ and $e_a$ are truly “intrinsic” (i.e., environment independent), the fitness of the four haplotypes when measured in the lab are given by $w_{AB} = (1 + s_{ab}/2)$, $w_{aB} = (1 - s_{ab}/2)$, $w_{AB} = w_{AB}(1 - e_A)$, and $w_{aB} = w_{aB}(1 + e_a)$.

To calculate the mean fitness of population $x$ (where $x$ may represent one of the source populations or a hybrid cross such as $F_1$ and $F_2$), we must know the genotype frequencies. These genotype frequencies can be calculated as $f_{AB,x} = p_{A,x}p_{B,x} + d_{x}f_{ab,x} = p_{A,x}(1 - p_{B,x}) - d_{x}f_{ab,x} = (1 - p_{A,x})p_{B,x} - d_{x}$, and $f_{ab,x} = (1 - p_{A,x})(1 - p_{B,x}) + d_{x}$, where $D_x$ is the linkage disequilibrium in population $x$. These values for the source populations are given above and in the supplementary material. For the $F_1$ hybrid population, $p_{A,F_1} = 1/2(p_{A,1} + p_{A,2})$ and $p_{B,F_1} = 1/2(p_{B,1} + p_{B,2})$. The linkage disequilibrium in the $F_1$ population is

$$D_{F_1} = \frac{1}{4}(1 - r)(m_1(s_1 + e_A) + m_2(s_2 + e_A + 2e_a)) + O(\xi^2).$$

Assuming future hybrid generations can be produced without selection, the allele frequencies of the $F_n$ hybrid generation remain the same as those in the $F_1$. The disequilibrium of later generation hybrids is given by $D_{F_n} = D_{F_1}(1 - r)^{n-1}$.

Intrinsic isolation is quantified by comparing the mean fitness of $F_n$ hybrids to the average of the mean fitnesses of individuals from each of the two parental populations:

$$I_{F_n} = \frac{1}{2}(E[w_{P1}] + E[w_{P2}]) - E[w_{F_n}],$$

where $E[w_{x}] = \sum f_{ix} w_{ix}$ is the mean fitness of population $x$. Evaluating this expression with the equilibrium genotype frequencies gives

$$I_{F_n} = (e_A + e_a)(\frac{1}{4} - D_{F_n}) + O(\xi^2).$$

This is always positive because $0 \leq D_{F_n} < 1/4$ indicating that hybrids are less fit than parental types. As expected, the degree of isolation depends on the intrinsic epistatic effects of the modifier, $e_A$ and $e_a$. The modifier allele $b$ causes the evolution of intrinsic isolation; $I_{F_n} = 0$ if $b$ is absent from the system, but $I_{F_n} > 0$ after $b$ invades. To leading order, this measure of intrinsic isolation is largely independent of any inadvertent ecological selection in the lab, $s_{ab}$, though higher order terms include interactions between ecological and intrinsic selection (see supplementary material). Similarly, the true ecological selection that initiated divergence ($s_1$, $s_2$) does not appear in (10). These parameters do have a weak effect on $I_{F_n}$ (see higher order terms shown in supplementary material) because they affect the degree of divergence between parental populations and thus the magnitude of disequilibrium in hybrids. While ecological selection does not have an important quantitative direct effect on the degree of intrinsic isolation, as measured in the lab, it does have a critical role. Ecological selection initiates the divergence between populations that allows the invasion of $b$ and the establishment of a stable equilibrium in which both loci are polymorphic. In principle, the effects of ecology could be discerned if lab rearing conditions were to properly emulate the divergent environmental selection pressures experienced by the parental populations in nature.

3.3. The Barrier to Gene Flow. In the previous section, we quantified the extent of intrinsic isolation as might be measured empirically. Intuitively, we would expect the evolution of intrinsic isolation to serve as a barrier to gene flow and, as such, contribute to speciation [14, 32]. Here we show that this is not always true.

We quantify the barrier to gene flow using the metric proposed by Zhivotovsky and Christiansen [52]. This measure reflects the disadvantage experienced by an unlinked neutral marker that enters a focal population through migration from the other population. In the absence of differentiation between the populations, a neutral marker that migrates into a population would have no disadvantage and the barrier strength would be zero. If the two populations are completely incompatible such that all hybrids die, then the barrier strength is infinite. The barrier is quantified as

$$B = -\ln\left(\prod_{t=0}^{\infty} \frac{w_{mt}}{w_{rt}}\right),$$

where $w_{mt}$ is the average fitness, in generation $t$, of the descendants of migrants that entered the population in generation $t_0$, $w_{rt}$ is defined analogously for a corresponding set of resident descendants. This measure of barrier strength is closely related to that suggested by Bengtsson [53] provided that the focal neutral locus is unlinked to the genes responsible for the barrier [54].

The analytical approximations given below are based on the first five terms in the infinite series in (11). This provides a reasonably good approximation because the effectiveness of the barrier quickly declines with each additional generation postmigration as the unlinked neutral marker disassociates from migrant genetic background in which it initially immigrated. The results presented below assume that recombination is strong relative to selection. Details on the calculation of the barrier, including results that allow for tight linkage, are provided in the supplementary material.

Taking Population 1 as our focal population, we find that the barrier to gene flow of alleles from Population 2 into Population 1 is

$$B_1 = \frac{31}{32} (s_1 - e_a) + \frac{1}{32} r(57 - 42r + 22r^2 - 7r^3 + r^4) \times (e_A + e_a) + O(\xi^2).$$

(12)
Several important points emerge from this result. Both ecological selection \( s_1 \) and intrinsic selection \( \varepsilon_A, \varepsilon_a \) affect the strength of the barrier. Whereas ecological selection clearly has a positive effect on the barrier, the same cannot be said of intrinsic epistatic selection. The second term in (12) indicates a positive effect of intrinsic epistatic selection on barrier strength but the first term shows a negative effect. These conflicting effects can be understood as follows.

Consider an unlinked neutral allele \( n \) entering Population 1 from Population 2 in an \( ab \) genotype (the common genotype in Population 2). For as long as the marker allele \( n \) remains associated with the \( a \) allele, it suffers from ecological selection against \( a \) in Population 1. The \( b \) allele provides an intrinsic advantage to \( a \)-bearing individuals \( (\varepsilon_a) \), thereby reducing the net selection against the \( ab \) genotype. By ameliorating the selection against \( a \), the \( b \) allele makes it easier for the neutral marker \( n \) to successfully migrate into the new population, thus reducing the barrier (see first term of (12)). However, recombination can separate the \( a \) and \( b \) alleles from each other such that the marker allele \( n \) may then find itself in the \( Ab \) genotype, which suffers negative intrinsic epistatic selection \( (w_{ab} - w_{Ab} \approx \varepsilon_A + \varepsilon_a) \). This breakdown of the coadapted gene complex makes it more difficult for the neutral marker to successfully introgress into the new population, thereby increasing the barrier strength (see second term of (12)).

The extent to which the marker will suffer from negative effects of the coadapted gene complex breakdown depends on the rate of recombination, \( r \), between the \( A \) and \( B \) loci. If the \( A \) and \( B \) loci are tightly linked (i.e., \( r \) is low), the unlinked marker will have left the \( ab \) haplotype before this haplotype is destroyed by recombination. Conversely, when \( r \) is high, the marker has a good chance of still being on the \( ab \) haplotype when recombination occurs between the \( A \) and \( B \) loci. This effect of recombination is reflected in the second term of (12) which shows that \( r \) mediates the negative effects of intrinsic epistatic effects on gene flow (or, equivalently, the positive effects on barrier strength). Tightly linked coadapted complexes serve as weaker barriers to gene flow at unlinked loci than loosely linked complexes. Gavrilets [32] previously showed that barrier strength increases with recombination for DM incompatibilities (see also Bengtsson [53]).

In the opposite direction, the leading order approximation for the barrier entering Population 2 from Population 1 is

\[
B_2 = \frac{31}{32} (s_2 + \varepsilon_a) + \frac{1}{32} r (57 - 42r + 22r^2 - 7r^3 + r^4) + O(\xi^2).
\]

(13)

This equation is similar to (12) except the first term which is different in two ways. First, the relevant ecological selection is that which occurs in Population 2 (i.e., \( s_2 \) rather than \( s_1 \)). Second, the intrinsic epistatic effect is positive in (13) rather than being negative as in (12). Just as the presence of \( b \) ameliorated the ecological disadvantage of \( a \)-bearing migrants competing against \( A \)-bearing residents in Population 1 (causing a negative effect on \( B_1 \)), the \( b \) allele exaggerates the ecological advantage of \( a \)-bearing residents in Population 2 competing against \( A \)-bearing migrants (i.e., \( w_{Ab} \) versus \( w_{ab} \) in Population 2). Combined with the second term, which reflects the consequences of breaking down the coadapted gene complex, the intrinsic epistatic effects always increase the barrier in Population 2.

The reason that Population 1, rather than Population 2, experiences conflicting effects of intrinsic isolation is because of the perspective we have taken here. In discussing the effect of intrinsic isolation on the barrier strength, we have implicitly compared the barriers when \( b \) is absent (and there is no intrinsic isolation) to the barriers after \( b \) invades. The strength of the barriers when \( b \) is absent is given by (12) and (13) with \( \varepsilon_A = \varepsilon_a = 0 \). The effects of intrinsic isolation on each population should be interpreted with respect to that population’s current genetic composition relative to its composition prior to the evolution of intrinsic isolation. Initially, Population 1 had the appropriate epistatic allele \( (B) \), whereas Population 2 did not. The evolution of intrinsic isolation increases the barrier strength for the population in which the new modifier allele is common (Population 2). However, the effect on the barrier in the other population (Population 1) may be positive or negative, depending on recombination.

3.4. Numerical Simulations. Numerical simulations were performed to test several of the major conclusions from analytical approximations presented above. The results are presented in Figures 2 and 3. Divergence at the \( A \) and \( B \) loci between the two populations is shown in Figures 2(a), 2(b), 3(a), and 3(b) for Cases 1 and 2 (full model), respectively. In each figure, the black line represents the level of divergence before the evolution of the \( b \) allele whereas the colored lines show divergence after the introduction of this allele. For both models, divergence between populations is only possible when migration is weak relative to selection (Figures 2(a), 2(b), 3(a) and 3(b)). Divergence at the \( B \) locus (i.e., evolution of the epistatic modifier \( b \)) typically has little quantitative effect on divergence at the \( A \) locus (compare colored lines to black lines in Figures 2(a) and 3(a)), provided polymorphism persists. However, as discussed in our analytical section above, the introduction of the epistatic modifier can completely destabilize the polymorphism, causing loss of divergence at both loci. This tends to occur when the epistatic modifier confers a large benefit to the \( a \) allele (i.e., \( \varepsilon_a \) large relative to \( s \)) and there is linkage between the \( A \) and \( B \) loci (compare left \([r = 0.1]\) and right \([r = 0.5]\) columns in Figures 2(a) and 2(b)). Direct ecological selection on the \( B \) locus helps to maintain divergence at both loci (compare Figures 2(a) and 2(b) with Figures 3(a) and 3(b)).

The strength of intrinsic isolation is shown in Figures 2(c) and 3(c) for Cases 1 and 2, respectively. As indicated by the points in black, there is no intrinsic isolation prior to the evolution of the \( b \) allele. However, the introduction of the epistatic modifier causes the evolution of intrinsic isolation in the face of gene flow. The strength of intrinsic isolation increases with the magnitude of epistatic effects on either background \( (\varepsilon_A \text{ and } \varepsilon_a) \) (see colored lines in Figures 2(c) and 3(c)). Additional simulations (not shown) confirm that divergent ecological selection on the \( B \) locus has little
Values correspond to $m = s^* \{1/100, 1/80, 1/60, 1/40, 1/20, 1/10, 1/8, 1/6, 1/4, 1/2, 1, 2\}$

(a) Divergence at A locus

(b) Divergence at B locus

(c) Intrinsic Isolation (based on $F_2$ hybrids)

(d) Barrier into Population 1

Figure 2: Continued.
quantitative effect on strength of intrinsic isolation, provided polymorphism persists. However, ecological selection can have a critical qualitative effect by allowing divergence to occur despite gene flow; intrinsic isolation is limited by the genetic differentiation at the interacting genes.

When measured by using early generation hybrids (e.g., $F_2$), intrinsic isolation is larger if A and B are unlinked rather than linked (compare left and right columns of Figures 2(c) and 3(c), noting scales differ). This is because coadapted gene complexes are more likely to be broken down in early generation hybrids if recombination is high. However, if intrinsic isolation is measured using very late generation hybrids, say $F_{100}$, then linkage has a smaller effect because linkage disequilibrium has been mostly eliminated in late generation hybrids even if recombination is low (not shown).

The strength of the barrier to gene flow into each population is shown in Figures 2(d), 2(e), 3(d), and 3(e) for Cases 1 and 2, respectively. The black line represents the barrier strength prior to the introduction of the $b$ allele. Differentiation at the A locus alone creates a barrier to gene flow because of divergent ecological selection on this gene. The evolution of $b$ always increases the strength of Barrier 2 (i.e., colored lines lie above black line). Both beneficial epistatic effects on the $a$-allele background ($\epsilon_a$) and deleterious epistatic effects on the A-allele background ($\epsilon_A$) strengthen the barrier (see Figure 2(e)). As predicted from the analytical approximations, the $b$ allele can either increase or decrease the strength of Barrier 1; $\epsilon_A$ increases this barrier, whereas $\epsilon_a$ decreases this barrier. For example, high values of $\epsilon_a$ greatly reduce the barrier (red and blue lines in Figure 2(d)), though these negative effects occur primarily when there is linkage between genes. As predicted analytically, epistatic effects increase barriers to a greater extent when there is loose linkage (compare left and right columns in Figures 2(d) and 2(e)). If the interacting genes (A and B) are closely linked, a neutral marker allele has a better chance of escaping a migrant chromosome before an adaptive complex (such as $ab$) is converted into a maladaptive complex (such as $Ab$). Thus, linkage between the interacting genes makes it easier for neutral markers to move between populations, as observed for past models that focused on barriers to gene flow per se [14, 32]. Finally, and unsurprisingly, it is clear that direct ecological selection on the B locus strengthens the barrier to gene flow (compare Figures 2(d) and 2(e) with 3(d) and 3(e)).

A comparison of the analytical approximations to simulation results is presented in the supplementary material (Figures S1 and S2). As expected, the analytical results provide good approximations under the conditions under which they were derived ($m \ll s$). The analytical approximations tend to underestimate the magnitude of intrinsic isolation and overestimate the barrier strengths. The deviations of the analytical approximations from the simulation results increase as migration becomes of the same magnitude as selection. The most obvious discrepancy occurs when the analytical results wrongly predict the two-locus polymorphism is stable. Instability is predicted to occur when migration is strong relative to selection. However, the analysis is based on a weak migration assumption, so it is not surprising that our analytically derived stability conditions are not very good at quantitatively predicting instability.
Values correspond to $m = s^* \{1/100, 1/80, 1/60, 1/40, 1/20, 1/10, 1/8, 1/6, 1/4, 1/2, 1, 2\}$

(a) Divergence at A locus

Values correspond to $m = s^* \{1/100, 1/80, 1/60, 1/40, 1/20, 1/10, 1/8, 1/6, 1/4, 1/2, 1, 2\}$

(b) Divergence at B locus

Values correspond to $m = s^* \{1/100, 1/80, 1/60, 1/40, 1/20, 1/10, 1/8, 1/6, 1/4, 1/2, 1, 2\}$

(c) Intrinsic Isolation (based on $F_2$ hybrids)

Values correspond to $m = s^* \{1/100, 1/80, 1/60, 1/40, 1/20, 1/10, 1/8, 1/6, 1/4, 1/2, 1, 2\}$

(d) Barrier into Population 1

Figure 3: Continued.
We have also tested whether our results are sensitive to the assumption of haploidy by performing a series of simulations for diploids. Results for these simulations are presented in the supplementary material (Figures S(3)–S(8)). Qualitatively, all of our major results apply to diploids, including the counter intuitive finding that the evolution of intrinsic isolation can reduce barrier strength. This is most likely to occur in diploid systems when negative epistatic effects are recessive (compare Figures S3(d), S6(d), and S9(d)). This type of gene action underlies the dominance theory of Haldane’s Rule [55] and is common in *Drosophila* incompatibilities [3, 7, 56].

4. Discussion

4.1. The Evolution of Intrinsic Postmating Isolation. Here, we presented a theoretical analysis of the role that divergent natural selection can play in generating intrinsic reproductive isolation during divergence with gene flow. Our objective was to assess the extent to which intrinsic reproductive isolation must be a by-product of genetic divergence in allopatri.

The typical DM incompatibility is often depicted as shown in Table 1 where fitnesses are not ecologically dependent. This representation has the characteristic property that two high-fitness genotypes (*AB* and *ab*) are connected by a ridge of high-fitness genotypes, so it is possible to move from one high-fitness genotype to the other by a series of more or less neutral substitutions. This type of representation is common in text books (Barton et al. [57, page 643]) and speciation models (see [14, 27, 32, 49], see also Table 2). Truly allopatric populations can diverge along neutral ridges in the adaptive landscape. However, such divergence will be severely impeded if there is gene flow both because migration directly homogenizes populations and because selection will oppose alleles that are incompatible with other alleles found in some parts of the range. Even if divergence occurs via drift in allopatri, the two-locus polymorphism will collapse once contact is reestablished in a two-patch model [32, 53]. Such DM incompatibilities only become stable when more complicated geographies, such as stepping-stone type models, are invoked (e.g., see [32]).

Alternatively, high fitness ridges may not be completely flat so that selection can drive the local fixation of alleles that have the potential to be involved in incompatibilities. However, when there is gene flow, such alleles are expected to quickly spread across a population as they are initially beneficial everywhere if there is no divergent ecological selection. In the face of gene flow, incompatibilities can only evolve if mutations at different interacting loci spread simultaneously in different parts of the metapopulation [36] or if new alleles interact with previously established incompatibilities [37].

Although it is intuitively obvious that the addition of divergent ecological selection will make it easier to establish incompatibilities in the face of gene flow, this possibility has been largely ignored in terms of formal models (but see [18]). Our simplest finding was that maintaining differentiation is the primary contribution of divergent selection to the evolution of intrinsic isolation. In particular, establishing genetic differentiation at ecologically important loci provides the appropriate setting for the subsequent evolution of epistatically interacting modifiers that can pleiotropically cause intrinsic reproductive isolation. This can happen in two ways. Either divergent ecological selection can act directly on the modifier locus, or not.

Divergent ecological selection acting directly on a modifier locus (Case 2) is the easiest way to maintain high levels of differentiation, even with strong epistatic effects. In the case where ecological selection does not act directly on the modifier locus (Case 1), there are limits to the strength of intrinsic isolation that can evolve. It is difficult for epistatic modifiers with strong effects to remain differentiated between populations, unless there is the right balance between the modifier’s positive effect on the
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other. In contrast, Orr’s result does not involve ecological
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genotype, thereby mitigating its ecological disadvantage in
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4.2. The Barrier to Neutral Gene Flow. An interesting
counterintuitive and somewhat unexpected result from our
analysis is that the same genes that cause intrinsic isolation
can reduce the barrier to gene flow. By improving the
intrinsic fitness of an ecologically maladaptive allele (a),
an epistatic modifier (b) reduces the net selection against
foreign genotypes, potentially making it easier for foreign
alleles to enter the focal population. For a related reason,
the epistatic modifiers typically have asymmetric effects on
the barriers to gene flow. The barrier into the population
containing the derived modifier allele (b) tends to be stronger
than the barrier into the other population. The derived
modifier allele’s beneficial epistatic effect on its preferred
background makes it easier for genotypes from its population
to invade the other population while at the same time making
it more difficult for the reverse direction of gene flow.

Our result about the asymmetric effects of epistatic
modifiers on barrier strengths seems related to asymmetric
effects with respect to intrinsic isolation in classic DM
incompatibilities. We found that the barrier from the
population containing the derived modifier allele into the
population with the ancestral allele tends to be weaker than
the reverse situation, whereas Orr [28] showed that derived
alleles are more likely to involve (cause) incompatibilities.
However, the underlying reasons for these two types of
“asymmetries” are very different. Our result is driven by
the derived modifier improving the intrinsic fitness of one
genotype, thereby mitigating its ecological disadvantage in
one habitat and augmenting its ecological advantage in the
other. In contrast, Orr’s result does not involve ecological
dependence and is driven by negative effects of the derived
allele in (hybrid) combinations that have gone untested by
selection in allopatry.

4.3. Implications for Empirical Studies of “Speciation Genes”.
Much empirical work has been dedicated to finding “speci-
ation genes” underlying reproductive isolation (for reviews
see [3, 5, 6, 58–61]). Most studies have centered on genes
underlying intrinsic postmating isolation, particularly in
hybrids between species of Drosophila. A major discovery
emerging from this work, particularly in animal systems, is
that genes underlying intrinsic postzygotic isolation often
show molecular signatures of positive selection [3, 9, 62–
64]. In one case, the gene “Oberdrive”, the mechanism of
selection appears to be intragenomic conflict rather than
environmental, ecologically based selection [8], and a similar
mechanism is suspected in other cases [7, 65]. In contrast,
genetic studies in yeast implicate ecological adaptation as
the driver of postmating isolation [47, 48]. Nevertheless,
in most cases of putative speciation genes, the mechanisms
of selection involved are not yet known. As discussed in
the introduction, there is empirical evidence from other
types of studies that ecological divergence can indeed drive
the evolution of intrinsic postmating isolation. Collectively,
these studies suggest that the theoretical scenarios treated
in our paper are plausible and that studies of speciation
genomes might consider whether the genes involved evolve via
divergent selection, perhaps in the face of gene flow.

5. Conclusions

Postzygotic isolation is often dichotomized as “intrinsic”
or “ecological/extrinsic”. However, many cases of the spe-
ciation process may involve both [3]. Divergent ecological
selection is well documented and can allow for genetic
differentiation despite gene flow [14, 18]. Moreover, adap-
tation to particular environmental challenges may often
come at some pleiotropic cost. This creates the potential
for epistatic modifiers that ameliorate such costs but that
may be incompatible on the original genetic background.
Under such conditions, divergent ecological selection can
be considered the catalyst for the evolution of intrinsic
isolation. However, the genes that contribute to intrinsic
isolation may have mixed effects on barrier to gene flow.
Thus, although divergent selection can thus promote the
evolution of intrinsic isolation, the overall importance of this
for the process of ecological speciation will depend on (1) the
degree to which this actually reduces gene flow and (2) the
extent and rate to which this occurs relative to commonly
documented scenarios where divergent selection drives the
evolution of premating or extrinsic barriers to gene flow
[3, 24, 25, 66].

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References


